The Journal of
CLINICAL
ENDOCRINOLOGY

VOLUME 11 JULY, 1951 NUMBER 7
Copyright 1951 by the Association for the Study of Internal Secretions

THE USE OF PROGESTERONE IN TREATMENT OF CANCER OF THE PROSTATE*

J. B. TRUNNELL, M.D.,**† B. J. DUFFY, JR., M.D.,§†
VICTOR MARSHALL, M.D., WILLET F. WHITMORE, M.D.
AND HELEN Q. WOODARD, PH.D.

From the Departments of Clinical Investigation and Special Biochemistry of the Sloan-Kettering Institute, and the Genito-Urinary Department of Memorial Hospital, New York, N. Y.

THERE is little doubt that certain alterations of the hormonal balance in patients with cancer of the prostate constitute a decidedly beneficial therapeutic procedure. Though the response of even advanced cases to castration or to estrogen administration is often a dramatic restoration to apparently normal health, relapse has been the rule after periods varying from months to years (1). For reasons to be discussed, we have undertaken a clinical evaluation of the effect of progesterone not only in patients in whom the disease has relapsed, but also in previously untreated cases. The following is a report based on treatment with progesterone or with anhydrohydroxy-progesterone of 19 patients with cancer of the prostate.

Received for publication December 6, 1950.

* Read at the Annual Meeting of the Association for the Study of Internal Secretions, San Francisco, California, June 23, 1950.

These investigations were supported by the Office of Naval Research, Contract No. N668-T01; The American Cancer Society; and The Damon Runyon Fund.

** Present address: M. D. Anderson Hospital, University of Texas Postgraduate School of Medicine, 2310 Baldwin Street, Houston 6, Texas.

† 1949–50 Damon Runyon Fellows in Clinical Cancer Research.

§ Present address: Atomic Energy Project, University of Rochester School of Medicine, Rochester 20, New York.

METHODS

With 1 exception, all patients herein reported were studied in cooperation with the Genito-Urinary service of Memorial Cancer Center or the Cornell Urological service at Bellevue Hospital. In all instances the diagnosis of cancer of the prostate was confirmed histologically. These 19 patients, ranging in age from 47 to 85 years, can be conveniently classified into three groups:

I. Those who had had no previous therapy of any kind and whose serum acid phosphatase levels were elevated.

II. Those who had had benefit from one or more of the conventional types of therapy and in whom the disease had relapsed *with* elevated serum acid phosphatase levels.

III. Those who had had benefit from one or more of the conventional types of therapy and in whom the disease had relapsed *without* elevation of the serum acid phosphatase levels.

There were 9 patients in group I, 6 in group II, and 4 in group III. In the latter two groups, all patients were considered to be refractory to further conventional treatment. Material used in therapy was obtained from two sources. The earlier cases were treated with an aqueous suspension of progesterone, which, although effective, resulted in several painful reactions at the sites of injection. With an improved preparation (smaller particles and abandonment of agar) such reactions diminished sharply, but remained a serious problem during prolonged therapy. In a majority of cases solutions in oil were utilized. Several patients were treated with highly concentrated solutions of 500 mg./cc. of progesterone in benzyl alcohol. This preparation likewise was insufficiently tolerated to permit prolonged usage. Doses of injected progesterone ranged from 25 to 500 mg. per day. Two patients were treated by implantation of pellets of compressed progesterone. One of these received 50 mg. of pellets totalling 300, 600, and 600 mg. on separate occasions, at intervals of two weeks. For reasons discussed subsequently, these doses were not repeated in other cases. The other pellet-treated patient was given 50 mg. at approximately one-month intervals.

Those patients who were treated with anhydrohydroxy-progesterone[1] received 25 mg. per day by mouth. One patient was given 100 mg. per day.

Serum acid and alkaline phosphatase measurements were made by a method described elsewhere (2). The upper limits of normal, by this method, are considered to be 1 unit of acid phosphatase and 5 units of alkaline phosphatase.

[1] Also called pregneninolone or ethinyl-testosterone.

RESULTS

The results of treatment are summarized in Table 1.

TABLE 1. RESPONSES TO ADMINISTRATION OF PROGESTERONE OR ANHYDROHYDROXY-PROGESTERONE TO 19 PATIENTS WITH PROSTATIC CANCER*

Stage of disease	Progesterone (25–500 mg. daily, i.m.)			Anhydrohydroxy-progesterone (25–100 mg. daily, by mouth)		
	Made worse	Made better	No effect, or difficult to evaluate	Made worse	Made better	No effect, or difficult to evaluate
Previously untreated	1	8	1	3	2	—
In relapse, after beneficial response to castration or to estrogen therapy	1	3	2	1	2	2

* Seven patients are listed in more than one category. These men either received both compounds (at different times) or exhibited more than one response (*see* text).

The acid phosphatase level in all but 1 (#92875) of the patients in groups I and II fell toward or into the normal range soon after treatment was started. This trend continued until about the third week, at which time (with 1 exception; Fig. 1, #93742) the minimum point was reached (Figs.

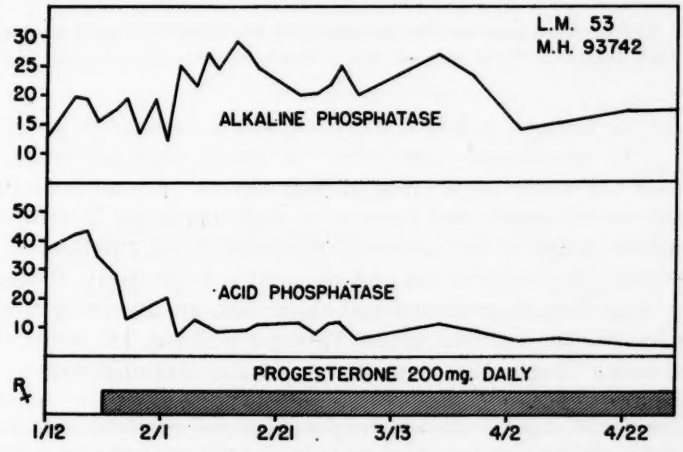


FIG. 1. Showing fall of acid phosphatase level and alkaline phosphatase "flare."

1, 2, 3). In 1 patient an alkaline phosphatase "flare" similar to that seen following castration or estrogen therapy was seen.[2]

Concomitantly, there occurred moderate to complete relief of pain, increase in appetite, and weight gain (in 2 instances amounting to 30 and 35 pounds). Two patients were relieved of symptoms of urinary obstruction, and 1 showed complete correction of marked anemia during the period of treatment. In 5 instances of interruption of therapy (usually because of local reaction to injections), there were reversals of favorable trends, *i.e.*, rise in acid and alkaline phosphatases, loss of weight, and return of pain, Fig. 4). Resumption of treatment, however, was followed by further periods of improvement.

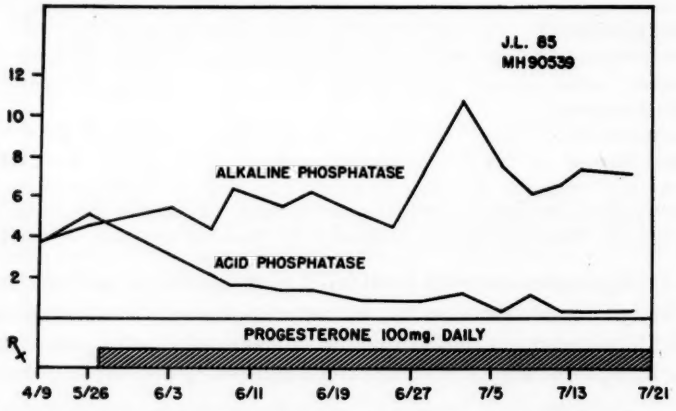


FIG. 2. The decrease in serum acid phosphatase was somewhat more gradual than in other patients. There was no well defined alkaline phosphatase "flare."

One of the Group I patients, #92875, failed to respond to progesterone (Fig. 5). He subsequently also failed to benefit from castration. These events are not surprising in view of both clinical (3) and laboratory (4) evidences which suggest that there is no single hormonal alteration which will similarly influence all cancers of the prostate. In 2 patients in Group I the disease relapsed after ten and six months respectively. Progesterone therapy was then discontinued and these men are currently being controlled by estrogen administration. Patients in Group III, while claiming to "feel better" for a short time after treatment was undertaken, did not

[2] During initial stages of favorable response, patients with bone metastases often show a rise in alkaline phosphatase before the level of this enzyme begins to fall. This is thought to be a function of bone regrowth in perimetastatic areas.

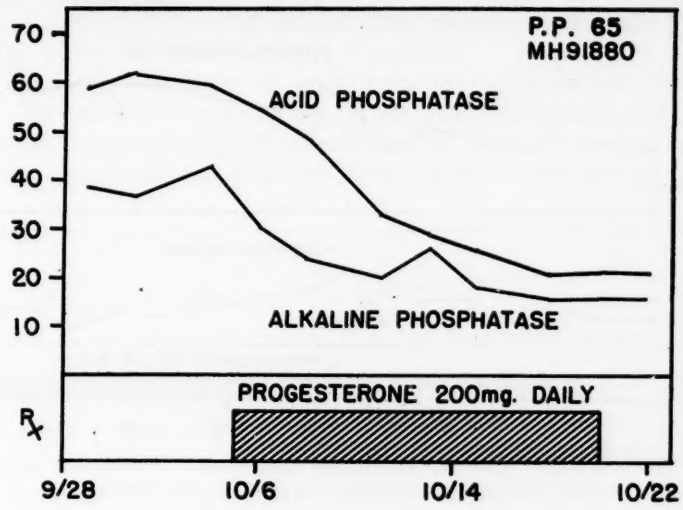


FIG. 3. This patient, who had failed to respond to large doses of estrogen, showed a prompt change when given progesterone.

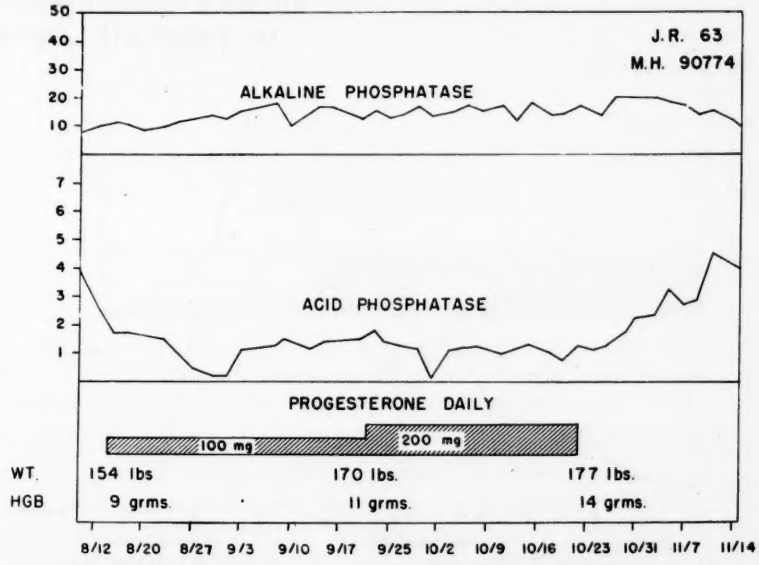


FIG. 4. The administration of progesterone was associated with a prompt fall in acid phosphatase, relief of pain, improvement in appetite, weight gain, and hemoglobin restoration in this previously untreated patient. These trends were reversed when progesterone was discontinued and again reversed when treatment was restarted (latter not shown).

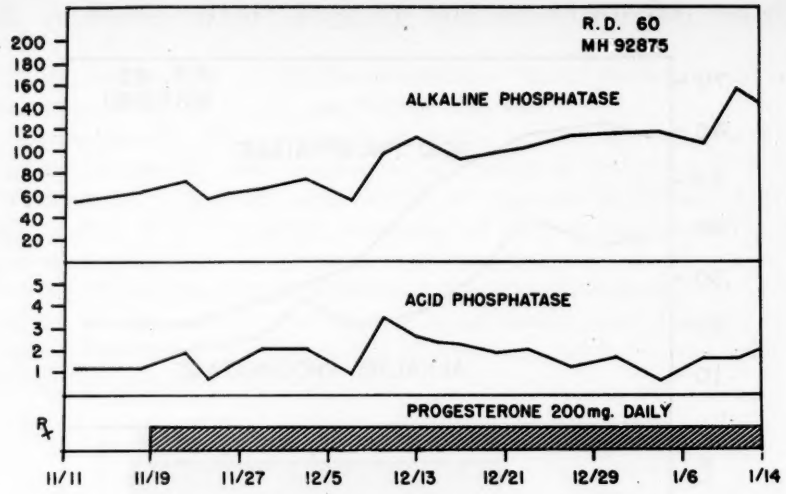


FIG. 5. This previously untreated patient did not respond to treatment with progesterone. Subsequent castration was likewise of no benefit.

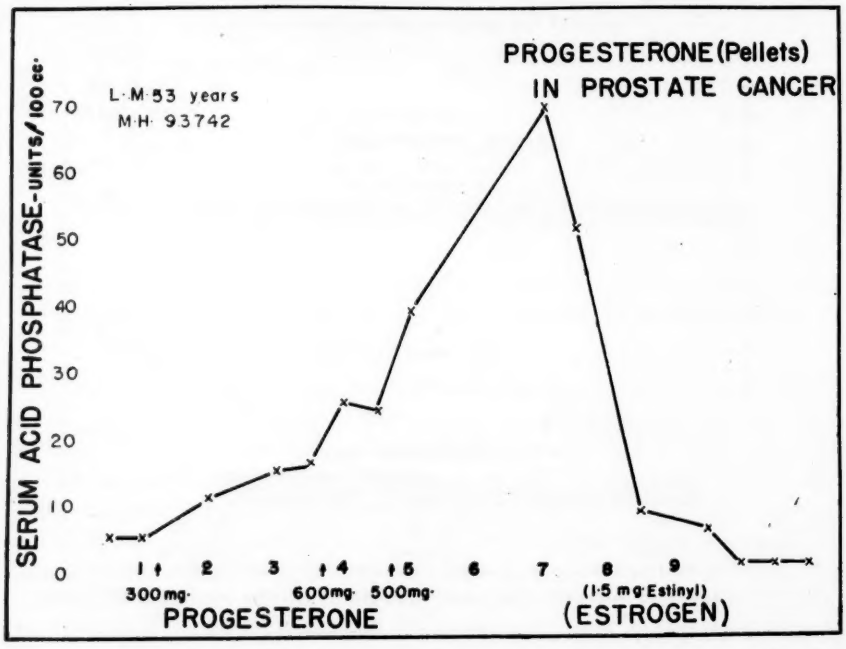


FIG. 6. Here are illustrated *increases* in serum acid phosphatase levels following implantation of progesterone pellets in a man previously controlled on 200 mg. of progesterone daily by injection. This is felt to indicate that the relative effectiveness per milligram of pellet administration is considerably greater than that of single daily injections.

exhibit objective evidence of change. The progress of their disease appeared to be neither accelerated nor decelerated.

Four patients who received anhydrohydroxy-progesterone were improved. Two of these were in Group I, previously untreated, and 2 were in Group II. Two of these 4 individuals received 25 mg. per day by mouth, 1 received 50 mg. per day, and the fourth was given 100 mg. per day by mouth.

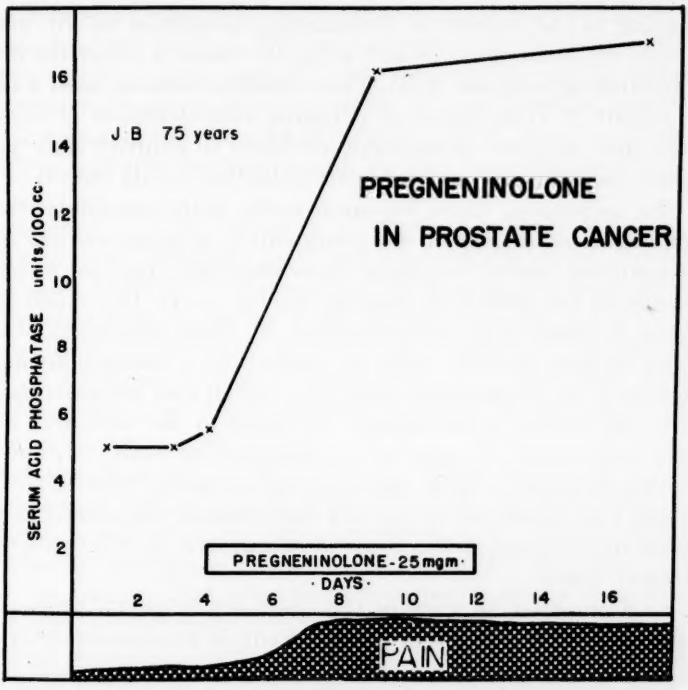


FIG. 7. Illustrating changes produced by pregneninolone (anhydrohydroxy-progesterone) in a patient already undergoing early relapse following beneficial responses to stilbestrol and to castration. The medication was administered orally.

Two patients given progesterone, and 4 who received anhydrohydroxy-progesterone, were made distinctly worse. In these instances pain and debility were intensified, and the serum acid phosphatase values rose steeply (Figs. 6, 7). These rather alarming trends were halted and reversed in 1 instance by administration of estrogen (Fig. 6). One man died (Fig. 7), possibly as a result of tumor stimulation, after eight days of anhydrohydroxy-progesterone administration (25 mg./day). In the remaining 4 instances of these therapeutically untoward but physiologically intriguing responses, cessation of treatment sufficed to restore the pretreatment status.

DISCUSSION

Progesterone was selected for therapeutic trial in the hope that it would provide a physiologic means of suppressing androgen production. Huggins (5) suggested that improvement following castration of "new" cases was a function of removal of androgen-producing tissues. He explained relapse on the basis of gradually increasing adrenal output of androgens, which eventually corrected the beneficial "deficiency" caused by orchiectomy.

According to the concept of Reifenstein, Albright *et al.* (6), androgen production by the adrenals as well as by the testes is under the influence of the luteinizing hormone (LH). These workers demonstrated a decrease in the output of 17-ketosteroids following administration of methyltestosterone and adduced considerable evidence to support their point of view that the decrease depended upon reduction of LH output.

On first inspection, there appeared to be little possibility that this concept might be utilized in the treatment of prostatic cancer. Methyltestosterone and related androgens have long been regarded as potential stimulators of the growth of prostatic cancer (5, 7). One could scarcely anticipate therefore that administration of these substances would be beneficial to such patients, even if a reduction in endogenous androgen supply were to be accomplished. However, we felt that progesterone, which decreases LH output in the female (8), might in the same way decrease androgen production in prostatic cancer-bearing males without itself stimulating the disease. Were such to occur (assuming reduction of androgen supply to *be* beneficial), it was felt that progesterone should be equally efficacious in new cases and in those in relapse, whether the patients were castrated or intact.

We were prepared, in view of the relatively unknown propensities of progesterone, to observe either improvement or accelerated deterioration in the patients chosen for initial treatment. It was not surprising therefore to observe both responses, not only in previously untreated patients, but also in those in whom the disease had undergone a spontaneous relapse. Further, there was general precedent (occasional failure to respond to castration to or estrogens) for the several patients who exhibited no measurable response to progesterone.

Measurements of LH excretion before and after progesterone administration are currently being made in an attempt to test the hypothesis which led to these trials. However, because of the divergent responses discussed in the foregoing paragraph, it may prove less profitable to be concerned with the validity of the primary hypothesis than to initiate study of the questions raised by its preliminary exploration.

Chief among the latter questions are these: 1. Which steroid hormones

may stimulate prostatic cancer? and 2. Which may act as inhibitors? The observation that progesterone and pregneninolone may make some of these patients worse enables one fairly to ask whether or not numerous other steroids might produce similar results. If so, testosterone may (as now thought) or may not be the most potent stimulator of prostatic cancer growth. By the same token it is conceivable that a variety of steroids (other than estrogens and progestins) may under appropriate circumstances also elicit other than their expected response when given to men with cancer of the prostate. We have recorded elsewhere (9) observations of our own and of others suggesting that this may be true; *i.e.*, testosterone may occasionally have either no effect or even produce improvement, whereas estrogens may, instead of being beneficial, have no effect or may actually be harmful. The latter possibility may be applied as well to castration.

Consideration of the foregoing possibility enabled us finally to ask whether or not androgens are really the culprits in the etiology and maintenance of prostatic cancer. Such a question would be asked with considerable trepidation were it not for the fact that Scott and his coworkers (10) have cautiously made a similar suggestion. The question takes on added significance in that their reasoning began with an entirely different premise (that the average testis produces less androgen and more estrogen as age increases).

Any attempt, however attractive, to evaluate the tenability of these novel points of view is beyond the scope of the present communication. Numerous other variables, not mentioned here, require simultaneous consideration in metabolic studies before an orderly concept of action can be even postulated. The issues have been raised simply to indicate that, regardless of the therapeutic suitability of progesterone in prostatic cancer, its trial has reopened a question too long neglected.

One final word is in order regarding the present status of progesterone in the treatment of prostatic cancer. Although efficacious, it cannot be recommended in preference to castration or to estrogen administration, if for no other reason than the relatively greater difficulty of administration which is involved. On the other hand, for patients in whom the disease has relapsed after other forms of treatment, a trial of progesterone would appear to be indicated.

We currently employ a daily intramuscular dosage of 100 mg. of progesterone in 80 per cent sesame oil and 20 per cent benzyl alcohol, a preparation relatively well tolerated. This is a more or less empirically chosen quantity but appears at the moment to be a reasonable starting point. In the event of apparent stimulation of the disease or of incomplete improvement, the dosage is decreased to 50 mg. per day and may be

reduced still further to 25 mg. per day if necessary. Doses larger than 100 mg. per day (up to 500 mg. per day) have not appeared to be of additional benefit and may result in stimulation of the cancer. We do not know whether injections given less frequently than once daily would be equally beneficial. Our experience with anhydrohydroxy-progesterone has been even more limited, and we can say with certainty only that this substance therapeutically is very definitely a two-edged sword. Doses of 50 mg. per day by mouth are suggested, again bearing in mind the possibility that a reduction in quantity may have to be effected. It is hoped that studies currently in progress may ultimately permit more definite recommendations regarding dosage. However, until the fundamental mechanism of the action of steroid regulation of prostatic cancer is more clearly understood, numerous individual variations will continue to make such recommendations both difficult and hazardous.

SUMMARY

In an attempt to reduce endogenous androgen production, 19 patients with carcinoma of the prostate gland have been given progesterone and/or anhydrohydroxy-progesterone.

1. Nine were previously untreated and of this number, 8 improved. The 1 who failed to do so also proved unresponsive to castration.

2. Ten patients in whom there had been a relapse of the disease following castration or estrogen therapy were similarly treated. Seven of these also showed varying degrees of improvement.

3. In 6 instances patients were made definitely worse as a result of these medications. Four of the 6 received anhydrohydroxy-progesterone.

4. Only 2 patients tolerated daily injections for long periods, hence little can be written at this time concerning duration of remissions so induced. With improved forms of dosage now available, we hope in the future to supply data relative to this question.

Acknowledgments

These studies have been carried out as part of an integrated program involving three institutions. Numerous individuals have contributed time, effort, knowledge, and materials for which we are most grateful and without which progress would have been slow indeed. It is a pleasure therefore to acknowledge our indebtedness to the following: Dr. Rulon W. Rawson, Chief of the Division of Clinical Investigation, for constant advice and criticism; Dr. E. C. Reifenstein, Jr., of Ayerst, McKenna, and Harrison, Ltd., not only for providing the aqueous suspension of progesterone with which these experiments were undertaken, but also for much valuable assistance and encouragement; Drs. Edward C. Henderson and Robert E. Waterman of the Schering Corporation for generously supplying large quantities of progesterone in oil and in a variety of other solvents in varying concentrations (they have also supplied ethinyl estradiol used in some of these cases); and Drs. Beecher Leversee and James Brady of Bellevue Hospital for cooperating

flawlessly in caring for several of the patients included in this report. We are likewise grateful to the Misses Anna Kuester and Willy Koetsier for technical assistance in carrying out the many phosphatase determinations required in this endeavor. The authors wish to thank Dr. Allister M. McLellan for permission to study and to report case A.G.

REFERENCES

1. Scott, W. W., and Benjamin, J. A.: A comparative follow-up study of the therapeutic value of bilateral orchiectomy in the treatment of carcinoma of the prostate, *J. Urol.* **6:** 604, 1948.
2. Woodard, H. Q.: Acid and alkaline glycerophosphatase in tissue and serum, *Cancer Research* **2:** 497, 1942.
3. Huggins, C., and Scott, W. W.: Bilateral adrenalectomy in prostate cancer: clinical features and urinary excretion of 17-ketosteroids and estrogen, *Ann. Surg.* **122:** 1031, 1945.
4. Horning, E. S.: The effects of castration and stilbestrol on prostatic tumors in mice, *Brit. J. Cancer* **3:** 211, 1949.
5. Huggins, C., and Hodges, C. V.: Studies on prostatic cancer. I. The effect of castration, of estrogen, and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate, *Cancer Research* **1:** 293, 1941.
6. Reifenstein, E. C., Jr.; Forbes, A. P.; Albright, F.; Donaldson, E., and Carroll, E.: Effect of methyl testosterone on urinary 17-ketosteroids of adrenal origin, *J. Clin. Investigation* **24:** 416, 1945.
7. Hovenian, M. S., and Deming, C. L.: The heterologous growth of cancer of the human prostate, *Surg., Gynec. & Obst.* **86:** 29, 1948.
8. Astwood, E. B., and Fevold, H. L.: Action of progesterone on gonadotrophic activity, *Am. J. Physiol.* **127:** 192, 1939.
9. Trunnell, J. B., and Duffy, B. J., Jr.: The influence of certain steroids on the behavior of human prostatic cancer, *Tr. New York Acad. Sci.* **12**: 238, 1950.
10. Scott, W. W.: Further hormonal relationships in prostatic growth. (Paper presented at Symposium of the New York Academy of Sciences, May 8, 1950.)

CASE SUMMARIES

J. R., *Mem. Hosp. No. 90774,* a 63-year-old white male, was first seen at Memorial Hospital in May, 1948, six months after the onset of back and leg pain. On admission he was found to have an enlarged, hard, right lobe of the prostate. A biopsy specimen obtained by aspiration showed carcinoma. X-ray examination revealed numerous metastases to pelvic and spinal bones. The average acid phosphatase value prior to therapy was 5 units; this began to decrease immediately when treatment with progesterone, 100 mg. intramuscularly each day, was begun. Pain diminished sharply, and the patient began to eat well and to gain weight. The acid phosphatase remained in the normal range for only two weeks and then rose to, and remained, only slightly (average 1.5 units) above the upper limit of normal. The progesterone dosage was doubled, and another brief drop to the normal zone was observed. Despite persistence of a slightly elevated acid phosphatase level, the patient continued to improve and in ten weeks had gained 23 pounds. During the same period his hemoglobin level had risen, without transfusion or antianemic therapy, from 9 grams to 14 grams per 100 ml. of blood. At this point therapy was discontinued in order to observe effects of withdrawal. The acid phosphatase immediately began to rise; the patient lost his appetite, had pain, and lost weight. After three weeks, treatment with progesterone was reinstituted, and all

previously observed beneficial effects were once again obtained. Treatment continued for ten months, at the end of which time anemia began to develop, pain and weight loss returned, purpura and subperiosteal hemorrhage developed, and the acid and alkaline phosphatase values rose. Progesterone was discontinued, and therapy with ethinyl estradiol 1.5 mg. per day was started. A new remission occurred which, at the time of writing, has lasted ten months. During the entire period of treatment no radiographic evidence of tumor regression was obtained.

J. L., *Mem. Hosp. No. 90539,* an 85-year-old white male, was first seen in the outpatient clinic of Memorial Hospital in April, 1948. He had had a suprapubic prostatectomy in 1935, but the microscopic sections from the prostate were not available. On admission he was found to have complete urinary obstruction, for which he had been catheterizing himself during a six-month period. Chronic cystitis had thereby been produced. The prostate area presented a stony, hard mass which on biopsy was reported to be adenocarcinoma. The serum acid phosphatase was elevated to 5 units, but the value for alkaline phosphatase was normal. X-ray examination revealed no metastases to bone. The acid phosphatase became and remained normal three weeks after progesterone therapy, 100 mg. per day intramuscularly, was started. The alkaline phosphatase slowly rose to slightly supernormal values. There was improvement in the urinary status, in that the patient could void spontaneously. It is conceivable, however, that at least some of this improvement stemmed from treatment of his cystitis.

The patient, who had senile mental changes, became acutely depressed over failure of members of his family to visit him, refused to eat, and became badly nourished. A jejunostomy was performed, but not soon enough to be of value. He died after the development of symptoms of urinary tract infection which did not respond to treatment. Postmortem examination disclosed renal infection with necrotizing papillitis; bronchopneumonia and carcinoma of the prostate invading the bladder and involving regional lymph nodes. Histologically the tumor showed no evidence of damage.

R. D., *Mem. Hosp. No. 92875,* a 60-year-old white male, was first seen at Memorial Hospital in August, 1948. Weakness and pain had developed eight months earlier. Biopsy of the prostate revealed an orderly adenocarcinoma. There were moderately extensive metastases to bone. Both acid and alkaline phosphatases were elevated above normal (2 and 45, respectively). He had had no therapy when progesterone, 200 mg. per day intramuscularly, was started November 19, 1948. There was no improvement by either clinical or laboratory criteria. The disease progressed steadily, although progesterone was continued for three months. One month after discontinuing the hormone, he was castrated. This procedure likewise did not prove beneficial, and the patient died three months later.

W. C., *Mem. Hosp. No. 87909,* a 59-year-old white male, was first seen at Memorial Hospital in October, 1947. The onset of symptoms had been in December, 1945, at which time he was admitted to another hospital where x-ray examination of the spine showed extensive osteoblastic metastases. The phosphatase levels were elevated (acid, 2.8 units and alkaline, 18 units), and a Papanicolaou urinary smear gave positive findings. An orchiectomy was performed January 26, 1946. Following the operation, treatment with stilbestrol was started. He remained essentially well until September, 1947, at which time there was recurrence of pain in the back and leg. At this time the acid and alkaline phosphatases again became elevated, the acid value averaging from 5 to 7 units. A course of urethane was begun in October, 1947, but was without benefit. In November,

1947, the patient was given x-ray therapy to the bone metastases with only slight relief. In December, 1947, a course of pituitary radiation was given. Throughout this time ethinyl estradiol was continued. Beginning January 16, 1948, a two-week course of progesterone, 50 mg. daily intramuscularly, was given with "remarkable diminution" in pain. He was discharged, receiving anhydrohydroxy-progesterone, 50 mg. per day by mouth, and tri-weekly injections of 50 mg. of progesterone. The serum acid phosphatase became normal (less than 1.0) unit after two weeks, but the alkaline value was essentially unchanged. The patient, who had been going downhill rapidly following all conventional therapy, was given four months of comfortable existence, during which time he was able to return to work. He had a severe relapse, however, in May 1948, deteriorated rapidly, and died. There was a rapid rise in acid and alkaline phosphatase levels terminally to 46.4 units and 46.7 units, respectively. Postmortem examination revealed widespread and viable tumor. There was no significant histologic change in any of the lesions despite the multiplicity of therapeutic attempts.

A. G., *N. Y. Hosp. No. 473226,* was a 69-year-old white male with widespread metastases to bone, from carcinoma of the prostate. He underwent transurethral resection and bilateral orchiectomy in April, 1947, and was started on stilbestrol therapy, 10 mg. a day. He remained quite well until December, 1948, at which time pain and weight loss recurred. In March, 1949, he weighed 132 pounds, had a red cell count of 3,800,000 and a serum acid phosphatase of 29 units. He was treated with anhydrohydroxy-progesterone, 100 mg. per day by mouth. After one week his pain had disappeared, and his appetite became enormous. After one month the acid phosphatase had fallen to 0.6 unit, the red count was 4,400,000, and he had gained nine pounds. He remained well for three months, and then had a relapse despite continuation of therapy.

J. B., *Mem. Hosp. No. 92138,* a 75-year-old white male, was first seen at Memorial Hospital in January, 1950. For two years he had had frequency and urgency. A transurethral resection was done in July, 1947, at which time an adenocarcinoma of the prostate was discovered. He was successfully treated with stilbestrol, 2 mg. per day. However, by August, 1948, he was once again confined to bed but made a brilliant recovery following orchiectomy. In late 1949 he once again suffered a relapse and was in considerable pain when first seen in our clinic. At that time the serum acid phosphatase value was 5 units, and that for the alkaline phosphatase was 76 units. Anhydrohydroxy-progesterone was given in doses of 25 mg. per day by mouth. After eight days there was a sharp rise in both acid and alkaline phosphatase values (16.5 and 164, respectively), and the patient had grown vastly worse. The medication was discontinued, but the patient expired ninety-six hours later.

L. M., *Mem. Hosp. No. 93742,* a 53-year-old white male, was first seen at Memorial Hospital in December, 1948. His first symptoms had been back and leg pain six months earlier. Nocturia (up to six times) developed, and he lost 25 pounds. When examined in our clinic the serum acid phosphatase was 71 units and the alkaline phosphatase was 21 units. He was treated with daily injections of 200 mg. of progesterone (January 22, 1949), whereupon he recovered appetite, strength and weight, and returned to work. The acid phosphatase level fell rapidly (two weeks) to 7 units. There was some fluctuation at approximately this same level, but the downward trend continued and by mid-June, 1949, was 2.8 units. During this period the patient was asymptomatic but objected to daily injections. An attempt was made to achieve the same effect by means of pellet implantations and on June 27, 1949, a total of 300 mg. (in six 50-mg. pellets) was implanted. In eight days the acid phosphatase had risen to 10.8 units. Feeling that the

amount given was inadequate, an additional quantity of 600 mg. was implanted. The acid phosphatase rose to 38 units and again pellets were implanted in a dose of 600 mg. (making a total of 1,500 mg.). The acid phosphatase rose to 74.4 units, and the patient's clinical condition became critical. A bleeding tendency and pneumonitis appeared, and the patient was unable to move because of pain. Therapy with ethinyl estradiol, 1.5 mg. per day by mouth, was started early in October and he made a rapid recovery, returning once again to his work. In May, 1950, the acid and alkaline phosphatase values were both normal. It is now felt that rather than being *inadequate*, the amount of progesterone implanted in pellet form actually constituted considerable over-treatment. It is of interest that, although the pellets were not removed, estrogen was nonetheless capable of inducing remission.

THE INFLUENCE OF CORTISONE AND ADRENOCORTICOTROPIN ON URINARY GONADOTROPIN EXCRETION*

ARTHUR R. SOHVAL, M.D. AND LOUIS J. SOFFER, M.D.

From the Endocrine Section of the Medical Services and the Chemical Laboratory, The Mount Sinai Hospital, New York, N. Y.

DURING a systematic investigation in our hospital of the effect of 17-hydroxy-11-dehydrocorticosterone (cortisone) and pituitary adrenocorticotropin (ACTH) in patients with various diseases, the clinical observations were supplemented by a large number of ancillary biochemical and physiologic studies. The major portion of these findings has been published elsewhere (1–5). The object of the present report is to record the results of assays for urinary gonadotropic activity in patients who were treated with these hormonal compounds.

MATERIAL AND METHODS

Included in this study are 22 patients with the following diseases: disseminated lupus erythematosus 11, hyperthyroidism 5, scleroderma 2, dermatomyositis 1, rheumatoid arthritis 1, acute rheumatic fever 1, and congenital hypoglobulinemia 1. Nineteen were females and 3 were males, each of the latter having disseminated lupus erythematosus. The ages of the subjects ranged from 13 to 52 years. However, there were only 2 women who exceeded 40 years of age, one aged 49 years and the other aged 52 years.

Cortisone was employed exclusively in 5 patients, ACTH in 10 and both in 7. In the last instance therapy with the two compounds was never simultaneous, ACTH being administered immediately on the cessation of cortisone therapy or after an interval of time. The usual initial daily dose of cortisone was 150 mg. and that of ACTH 100 mg. in 4 equally divided doses. In every instance treatment was continuous for at least one week, and often longer, with the initial full dose. Dosages were often subsequently reduced by gradual decrements whenever permitted by the clinical response. The duration of therapy varied approximately from two weeks to many months and was continuous, on a maintenance basis, in the patients with disseminated lupus erythematosus.

Received for publication November 30, 1950.

* Aided by a grant from the U. S. Public Health Service.

Assays for urinary gonadotropin were usually performed at weekly intervals on immature female white mice weighing 6 to 9 grams, according to the method of Klinefelter, Albright and Griswold (6) with certain modifications. Instead of employing a 90-minute aliquot of urine, one-third of a 24-hour icebox-stored collection was utilized. Uterine enlargement, the criterion of a positive response, was considered to be present only if the weight of the uterus was 10 mg. or more. In our laboratory the uteri of control mice occasionally weigh 8 or 9 mg. For this reason the critical 7-mg. endpoint of Klinefelter and his co-workers was not employed. This procedure, with us as well as with others (7), establishes a normal range of urinary gonadotropin excretion of 5 to 40 mouse uterine units per twenty-four hours. The present study is based on the results of 110 assays with 207 dose-levels of urinary extract.

Early in the progress of this work it became apparent that the urine of treated patients was frequently toxic to the mice. Efforts were made to eliminate or reduce the toxicity of the urinary extracts by dialysis but this procedure often resulted in a loss of gonad-stimulating activity. This was demonstrated after dialysis of nontoxic, potent extracts. Urinary extract toxicity was encountered over a wide range of dose volumes from 0.4 cc. (24 m.u.u.) to as little as 0.05 cc. (200 m.u.u.). It was more frequently observed at the lower mouse-unit levels (larger doses of extract) and rarely at the higher levels. Because of the lethal property of a considerable number of urinary extracts the results of many assays were not obtainable. This technical difficulty was responsible for two obstacles in the study. In the first place, it was often impossible to ascertain gonadotropin titers which may have been at subnormal, normal or even moderately elevated levels. Secondly, the absence of data on certain crucial days interrupted the continuity of the observed results. However, it can be seen that the aforementioned procedural impediments did not interfere significantly with the demonstration of well-defined increases in urinary gonadotropic activity.

RESULTS

In 9 of the 22 patients an excessive quantity of urinary gonadotropin was demonstrated during therapy (Table 1). Excessive urinary titers appeared in 5 patients (Cases 2, 3, 4, 5 and 9) during cortisone treatment and in 4 others (Cases 1, 6, 7 and 8) during the administration of ACTH. In 2 patients (Cases 2 and 9) increased urinary gonadotropins were demonstrated during therapy with ACTH which followed directly after an initial course of cortisone. One patient (Case 5) showed a rise in urinary gonadotropic activity during each of two separated courses of cortisone and one of ACTH. Another subject (Case 4) also excreted increased

TABLE 1. URINARY GONADOTROPIC ACTIVITY IN PATIENTS TREATED WITH CORTISONE AND ACTH

Case No.	Sex	Age (yrs.)	Disease	Urinary gonadotropins, m.u.u. per 24 hrs.*	Day of assay**	Dosage at time of assay, total dose (avg. daily dose)		Remarks
						Cortisone (mg.)	ACTH (mg.)	
1	F	13	Disseminated lupus erythematosus	<21	Pretreatment			Died on 10th day
				90	4		425 (105)	
2	M	51	Disseminated lupus erythematosus	<60	Pretreatment			
				90–180	7	1000 (145)		Cortisone for 16 days, then ACTH
				204	28	1925 (120)	725 (60)	
				<94	43		1185 (45)	
3	F	39	Disseminated lupus erythematosus	<21	Pretreatment			
				184–276	12	1200 (100)		
				<46	20	1945 (95)		
				<40	30	3145 (105)		
4a	F	32	Disseminated lupus erythematosus	60	11	2200 (200)		Therapy for 13 days
				75–150	15	2600 (175)		
				<28	20			
4b				135	5	500 (100)		Second course of therapy
				208	15	1050 (70)		
				<84	22	1575 (70)		
				<40	29	2225 (75)		
5a	M	13	Disseminated lupus erythematosus	198	7	1050 (150)		Therapy for 7 days
5b				<24	Pretreatment			
				60	4	600 (150)		Second course of therapy
				90	7	1050 (150)		
5c				180	7		425 (60)	Third course of therapy
6	F	28	Hyperthyroidism	<45	Pretreatment			
				90–180	5		500 (100)	
				<45	10		1000 (100)	
7	F	32	Hyperthyroidism	<60	Pretreatment			
				90–180	5		500 (100)	
				151–302	10		1000 (100)	Therapy for 10 days
				180	17			
				90–180	21			
8	F	18	Congenital hypoglobulinemia	180	2		125 (65)	
				180	5		425 (85)	
9	F	21	Scleroderma	<26	Pretreatment			
				90–180	7	965 (140)		Cortisone for 21 days, then ACTH
				240	14	1665 (120)		
				240	23	2365 (115)	125 (65)	
				<120	30		375 (55)	

* Highest level tested, except where indicated by a negative assay at a higher level to establish a range.
** Counting from the first day of treatment.

amounts of gonadotropin during two separated courses of cortisone therapy.

In 3 patients (Cases 4, 5 and 8) assays for urinary gonadotropin prior to treatment were not obtained. These subjects included an extremely ill woman of 32 years with acute disseminated lupus erythematosus, a moderately ill boy of 13 years with the same disease and an 18-year-old girl with edema due to congenital hypoglobulinemia. Although the pretreatment urinary gonadotropin titer of these patients is unknown, the relative youth of the subjects and the nature of their diseases would suggest normal or subnormal levels. Moreover, in the light of the pretreatment findings in the other patients of the entire group it is reasonable to assume that no elevation was present in the untreated state. Therefore, the demonstration of increased urinary gonadotropic activity during therapy in these 3 patients is regarded as genuine and significant. In the remaining 6 subjects there was an unequivocal rise in the urinary gonadotropin content during treatment with cortisone or ACTH. The extent of the increase in the levels of urinary excretion ranged from 60 to 240 mouse uterine units per twenty-four hours.

The 9 patients in whom the urinary excretion of gonadotropin was increased during or after treatment had the following diseases: disseminated lupus erythematosus 5, hyperthyroidism 2, congenital hypoglobulinemia 1, and scleroderma 1. Seven were females ranging between 13 and 39 years of age. Two were males, aged 13 and 51 years. The increase in the titer of urinary gonadotropin in 7 of the 9 cases appeared within the first week of treatment. In the remaining 2 patients (Cases 3 and 4) it was present on the twelfth and eleventh days respectively, no post-treatment assay having been performed prior to these days. The earliest observed appearance of an increase in the urinary gonadotropic activity following the institution of treatment occurred after two days during which a total dose of 125 mg. of ACTH had been administered. This occurred in an 18-year-old girl with congenital hypoglobulinemia (Case 8). In another patient (Case 1), a 13-year-old girl acutely ill with disseminated lupus erythematosus, an excessive titer of urinary gonadotropin was noted after she had received 425 mg. of ACTH over a 4-day period. In each instance in which an increased titer was demonstrated, it was present on the first technically satisfactory assay.

The question as to how long the increased urinary gonadotropin excretion persisted was not readily answerable for several reasons. First, the frequent toxicity of urinary extracts for the assay animals often interfered with serial observations. Second, some patients were not followed long enough with periodic assays. Finally, in 1 patient (Case 1), convulsions developed after eight days of therapy with ACTH and she died two days

later. Hence, the data derived from the present study preclude the formation of valid conclusions regarding the duration of the observed increase. Nevertheless, the available data are of value in suggesting certain generalizations in this connection. In 1 patient (Case 7) who received 100 mg. of ACTH daily for ten days, an elevated titer of urinary gonadotropin was still present seven and eleven days after cessation of therapy. In another patient (Case 4) who received 2.6 grams of cortisone over a 13-day period, the rise in urinary gonadotropin was absent one week later. Still another variation was encountered in a third patient (Case 6) in whom an increased titer which was noted on the fifth day had disappeared on the tenth day of treatment with ACTH. The disappearance or significant reduction of increased urinary gonadotropic activity while the patient continued under treatment was particularly striking in those individuals who were treated for more than two weeks. In 2 patients (Cases 3 and 4) the increased gonadotropic titer which was present at the end of two weeks was absent at the end of the third and fourth weeks. In 2 other instances (Cases 2 and 9), an increased titer which had persisted for four and three weeks was either absent or sharply diminished at the end of six and four weeks respectively. All 4 of these patients received continuous therapy with gradually decreasing daily doses.

Of interest are observations in 2 patients with disseminated lupus erythematosus who received more than one course of therapy. The periods of treatment were separated by time intervals which were long enough to permit the increase of urinary gonadotropin to subside. In each patient the lapse of treatment was associated with a recrudescence of the disease. A second course of cortisone in Case 4 was accompanied by the reappearance of excessive urinary gonadotropic activity on the fifth day of treatment. It was still present on the fifteenth day but was considerably reduced a week later and absent after a further week of treatment. In the case of the 13-year-old boy (Case 5) a second course of cortisone similarly resulted in the reappearance of an increased urinary gonadotropic content during one week of treatment. Three weeks after the termination of the second course of cortisone therapy, a course of ACTH was instituted. After one week of treatment a significant rise in urinary gonadotropic activity was again noted.

Careful study of the collected clinical observations and laboratory data of the 22 patients comprising this study failed to indicate why cortisone and ACTH increase the urinary gonadotropic activity in some patients and not in others. The presence or absence of this finding in any given patient could not be correlated with such factors as race, age or sex of the patient, the nature, duration or acuteness of the disease, the type, dosage or duration of therapy or the clinical response of the patient to the treat-

ment. Furthermore, there was no correlation with laboratory data derived from a large assortment of studies dealing with the metabolism of steroids, electrolytes, protein and carbohydrates.

DISCUSSION

The observed increase in the urinary excretion of gonadotropin soon after the beginning of therapy may represent a compensatory effect. However, the markedly increased titers, at times reaching levels ordinarily observed in castrates, do not appear to be compatible with this concept. Furthermore, the duration of the excessive excretion for periods of three (Cases 7 and 9) and four (Case 2) weeks is hardly consistent with the transitory character of compensatory or rebound phenomena. It is therefore unlikely that this mechanism contributes significantly to the demonstrated increase in urinary gonadotropic activity.

Adequate investigations of the effect of ACTH and cortisone on the gonadotropic potency of the urine have not been reported previously. Mason and his co-workers (8) administered 25 mg. of ACTH daily for six days, 50 mg. daily for the next six days, none for the following five days and then 100 mg. daily for eleven and one-half days to a healthy 32-year-old woman. She was single and had been menstruating regularly. The urinary excretion of gonadotropin, determined by the method of Frank (9), was found to be normal throughout the study. During the course of an extensive study of the physiologic effects of cortisone and ACTH on a group of more than 30 patients, Sprague and his collaborators (10, 11) refer to observations made in a 30-year-old woman receiving 100 mg. of cortisone daily. It is stated that the urinary gonadotropins remained normal during two menstrual cycles. Urinary gonadotropins were estimated by the method of Frank and his colleagues (12). It is not clear from these reports as to whether assays were performed during the early or later stages of treatment.

The discrepancy between the results reported in the 2 cases in the literature and those observed in the present study may be more apparent than real. In the first place the occurrence of an increased urinary gonadotropin titer in only 9 of our 22 treated patients indicates that this effect does not invariably occur. It need not necessarily be encountered, therefore, in very small groups of patients. Secondly, the results of the present study suggest that when ACTH or cortisone induces increased urinary gonadotropic activity it does so within the first two weeks of therapy. The increased activity may disappear during continued treatment. These effects, therefore, may escape detection unless assays are performed within the first week or two after institution of therapy. Finally, the dosage of the administered hormone may be of importance in relation to its

effect on urinary gonadotropin. The dose employed by Mason and his co-workers (8) is less than that administered in the present group of patients.

The appearance of increased urinary gonadotropic activity in patients treated with cortisone or ACTH is contrary to what one would expect in the light of other recognized effects of these compounds. Thus, the endogenous output of ACTH by the adenohypophysis is suppressed by both ACTH and cortisone. Similarly, there is evidence to indicate that these agents inhibit the secretion of thyrotropin by the adenohypophysis (13). It would have been reasonable to assume that the gonadotropic secretory function of the anterior pituitary would also be decreased by cortisone and ACTH. That the reverse effect occurs, at least as determined by the urinary excretion, is not as surprising as would appear at first glance. Relatively recent medical literature contains case reports in which adrenal cortical tumors or adrenal cortical hyperfunction were associated with excessive excretion of urinary gonadotropin. These cases are of particular interest in that they represent conditions which are somewhat analogous to those existing in the patients in the present experimental study.

McCullagh and Cuyler (14) described the instance of a 17-year-old girl (Case 11) with typical Cushing's syndrome and positive findings with the Friedman test. The urinary content of gonadotropin was again found to be elevated after denervation of the left adrenal gland was performed in an effort to alleviate the symptoms. There was no change in the clinical picture until a similar operation was performed on the right gland in conjunction with hemiadrenalectomy. This resulted in a marked clinical improvement and the Friedman test response became negative. The patient died some time later of an acute infection. An adenoma occupying two thirds of the anterior lobe of the hypophysis was found at necropsy. This was "predominantly chromophobic in type with possibly basophilic and eosinophilic cells." Adrenocortical hyperfunction in this patient was associated with the urinary excretion of excessive quantities of gonadotropin.

Additional pertinent clinical data include 3 patients with pathologically proven carcinoma of the adrenal cortex associated with excessive urinary gonadotropic activity. All were males less than 40 years of age and 2 were feminized. In 1, the feminization and positive response to the Friedman test disappeared after resection of the adrenal tumor. The cases have sufficient bearing on the text of the present discussion to warrant a brief summary.

McFadzean (15) reported the case of a 29-year-old man with carcinoma of the adrenal cortex and feminization characterized by gynecomastia

and decreased libido. Urinary gonadotropins were not measured quantitatively but were increased sufficiently to give a positive result with the Friedman test. Twenty days after resection of the tumor the Friedman test response was negative. By the forty-third postoperative day, sex interest and libido improved, erections were frequent and the beard was heavier. The gynecomastia was unchanged.

Reifenstein (16) cited a man in his early twenties with well defined Cushing's syndrome, including gynecomastia, associated with an excessive excretion of gonadotropin in the urine. The amount excreted was approximately 50 times the normal. A large quantity of gonadotropin was also found in the tumor by direct assay. It was not determined whether the tumor was the source of gonadotropin production or whether it merely served to store it after it was produced elsewhere.

A third case of adrenal cortical carcinoma in a male, associated with excessive quantities of urinary gonadotropin was reported by Chambers (17). This 39-year-old patient lacked convincing evidence of feminization, thereby differing from the cases of McFadzean and Reifenstein. The Friedman test findings were positive on 2 occasions. Quantitative assay yielded 50,000 m.u. of prolan A per 100 cc. of urine.

Further evidence of a correlation between adrenocortical hyperfunction and the urinary excretion of increased amounts of gonadotropin is suggested by the experience of Escamilla (7). In a group of 13 youthful patients with an adrenogenital syndrome he noted that "apparently, a slight increase in FSH is seen in about half of these cases."

Relevant to the present discussion would be observations on the urinary excretion of gonadotropins in a large number of patients with Cushing's syndrome. However, available reports are meagre and conflicting. These have been summarized by Luft (18) who cites instances in which urinary gonadotropins were increased and others in which no increase could be demonstrated. He points out that most of these reports are based on unreliable qualitative methods of determination and are therefore only of limited value. In his own experience, employing acceptable quantitative methods, only 1 of 14 patients with Cushing's syndrome excreted an increased amount of urinary gonadotropin. In 7 individuals, in whom a satisfactory assay for low titers could be obtained, a decrease was noted.

It is thus apparent that increased urinary gonadotropic activity may at times be encountered in states of adrenocortical hyperfunction regardless of whether the latter occur spontaneously as a result of disease or are induced by hormonal therapy. In either case the homeostatic disturbance seems to depend upon the presence of an excessive amount of one or more adrenal cortical hormones. From the present state of our knowledge it is not possible to predicate the mechanism by which adrenocortical hyper-

function results in increased urinary gonadotropic activity. As stated earlier, it was anticipated that excessive amounts of adrenal cortical hormones would inhibit adenohypophyseal gonadotropic secretion. This may occur under certain circumstances and in fact, is the generally accepted mechanism for the production of amenorrhea in Cushing's syndrome. It must be emphasized, however, that this concept has not been conclusively substantiated. Furthermore, data derived from animal experiments suggest that gonadotropic secretion by the adenohypophysis is not inhibited by cortisone. Employing ovariectomized immature rats united in parabiosis with intact littermates, Byrnes and Shipley (19) were able to show that cortisone, administered for ten days in daily doses ranging from 0.5 to 2 mg., failed to inhibit adenohypophyseal gonadotropic secretion. Although more extensive investigations in the human subject as well as in the experimental animal are required to elucidate this adrenocortical-adenohypophyseal relationship, some of the evidence already at hand points to the need for a revision of current hypotheses.

The nature of the substance responsible for the increased gonadotropic activity in the urine of certain patients treated with cortisone and ACTH is not known. Several possibilities can be entertained. First, that it is one or more natural gonadotropins derived from the adenohypophysis. Second, that it is a non-pituitary agent possessing a direct gonad-stimulating property. In this event it may be an adrenal cortical hormone or one of its metabolic derivatives. That it is probably not a direct effect of excreted cortisone is suggested by the studies of Antopol (20). This worker observed inhibition, rather than stimulation of the ovaries of immature mice given 1.25 mg. of cortisone daily for six days. No significant ovarian changes were noted in our laboratory when 1 mg. daily of cortisone was injected in immature mice over a period of two and one-half days. Finally, to be considered, is the possibility that the substance is metrotropic rather than gonadotropic thus resulting in positive findings when the uterine-weight method is employed. Such a mode of action can be excluded by assaying potent urinary extracts in ovariectomized mice. This was performed with 3 separate extracts which gave positive results (increased uterine weight) in intact mice, but negative results in spayed mice. It must, therefore, be concluded that the responsible urinary agent is a true gonadotropin. For the present, however, it cannot be stated whether or not it is of adenohypophyseal origin.

SUMMARY

1. Urinary gonadotropin assays were performed in a group of 22 patients who received cortisone or pituitary adrenocorticotropin therapy.

2. Excessive urinary gonadotropic activity was demonstrated in 9 of

the 22 patients during treatment. It occurred about as often with cortisone as with ACTH and its presence could not be correlated with the race, age or sex of the patient, the nature, duration or acuteness of the disease, the type, dosage or duration of therapy or the clinical response of the patient to the treatment.

3. The increased urinary gonadotropin titer appeared within the first week of treatment in 7 of the 9 cases. In the remaining 2 patients it was noted on the eleventh and twelfth days respectively, no post-treatment assay having been performed earlier.

4. The duration of the increased urinary gonadotropin levels was quite variable. In one patient it disappeared by the tenth day of treatment, whereas it was still present eleven days after a ten-day period of treatment in another patient. Particularly noteworthy was the disappearance or significant reduction of increased levels while the patient received continued therapy for more than two weeks.

5. In 2 patients multiple courses of treatment resulted each time in the reappearance of increased urinary gonadotropin.

6. The mechanism by which cortisone and other adrenal cortical hormones stimulated by ACTH increase urinary gonadotropin excretion is not known. Clinical records are cited from the literature to support a correlation between adrenocortical hyperfunction and elevated urinary gonadotropin. The data at hand do not indicate whether or not the excessive quantity of excreted gonadotropin is of adenohypophyseal origin.

REFERENCES

1. Baehr, G., and Soffer, L. J.: Treatment of disseminated lupus erythematosus with cortisone and adrenocorticotropin, *Bull. New York Acad. Med.* **26**: 229–234 (April) 1950.
2. Soffer, L. J.; Boas, N. F.; Levitt, M. F.; Gabrilove, J. L., and Baehr, G.: The control of disseminated lupus erythematosus and other diseases of mesenchymal tissues by cortisone and adrenocorticotropin, *Tr. A. Am. Physicians* (in press).
3. Soffer, L. J.; Levitt, M. F., and Baehr, G.: The influence of cortisone and adrenocorticotropin in acute disseminated lupus erythematosus, *Arch. Int. Med.* **86**: 558–573 (Oct.) 1950.
4. Boas, N. F., and Soffer, L. J.: The effect of adrenocorticotropic hormone and cortisone on the serum hexosamine level in acute disseminated lupus erythematosus, *J. Clin. Endocrinol.* **11**: 39–45 (Jan.) 1951.
5. Reiner, M.: Effect of cortisone and adrenocorticotropin therapy on serum proteins in disseminated lupus erythematosus, *Proc. Soc. Exper. Biol. & Med.* **74**: 529–531 (July) 1950.
6. Klinefelter, H. F., Jr.; Albright, F., and Griswold, G. C.: Experience with a quantitive test for normal or decreased amounts of follicle stimulating hormone in the urine in endocrinological diagnosis, *J. Clin. Endocrinol.* **3**: 529–544 (Oct.) 1943.

7. Escamilla, R. F.: Diagnostic significance of urinary hormonal assays: report of experience with measurements of 17-ketosteroids and follicle stimulating hormone in the urine, *Ann. Int. Med.* **30:** 249–290 (Feb.) 1949.
8. Mason, H. L.; Power, M. H.; Rynearson, E. H.; Ciaramelli, L. C.; Li, C. H., and Evans, H. M.: Results of administration of anterior pituitary adrenocorticotropic hormone to a normal human subject, *J. Clin. Endocrinol.* **8:** 1–14 (Jan.) 1948.
9. Frank, R. T.: A modification of the Zondek alcohol method for determining both luteinizing and follicle stimulating principles in the urine, *Endocrinology* **25:** 996–997 (Dec.) 1939.
10. Sprague, R. G.; Power, M. H.; Mason, H. L.; Albert, A.; Mathieson, D. R.; Hench, P. S.; Kendall, E. C.; Slocumb, C. H., and Polley, H. F.: Observations on the physiologic effects of cortisone and ACTH in man, *Arch. Int. Med.* **85:** 199–258 (Feb.) 1950.
11. Sprague, R. G.; Power, M. H., and Mason, H. L.: Observations on the physiological effects of cortisone and ACTH in man, *Recent Progr. Hormone Research* Vol. **6** (in press).
12. Frank, R. T.; Salmon, U. J., and Friedman, R.: Determination of luteinizing and follicle-stimulating principles in castrate and menopause urine, *Proc. Soc. Exper. Biol. & Med.* **32:** 1666–1667 (June) 1935.
13. Soffer, L. J.; Gabrilove, J. L., and Jailer, J. W.: Role of adrenal in uptake of I^{131} by the thyroid following parenteral administration of epinephrine, *Proc. Soc. Exper. Biol. & Med.* **71:** 117–119 (May) 1949.
14. McCullagh, E. P., and Cuyler, W. K.: The Friedman test and pituitary tumor, *Endocrinology* **21:** 8–18 (Jan.) 1937.
15. McFadzean, A. J. S.: Feminization associated with carcinoma of the adrenal cortex, *Lancet* **2:** 940–943 (Dec. 28) 1946.
16. Reifenstein, E. C., Jr.: Discussion of article by Soffer, L. J., Gabrilove, J. L., Jailer, J. W., and Jacobs, M. D.: The virilizing syndrome in man, *Recent Progr. Hormone Research* **5:** 407–428, 1950.
17. Chambers, W. L.: Adrenal cortical carcinoma in a male with excess gonadotropin in the urine, *J. Clin. Endocrinol.* **9:** 451–456 (May) 1949.
18. Luft, R.: A study on hirsutism, Cushing's syndrome and precocious puberty, *Acta med. Scandinav.* Supp. 149, pp. 1–119, 1944.
19. Byrnes, W. W., and Shipley, E. G.: Estrogenic and gonadotropic hormone inhibiting activity of some adrenal cortical substances, *Proc. Soc. Exper. Biol. & Med.* **74:** 308–310 (June) 1950.
20. Antopol, W.: Anatomic changes produced in mice treated with excessive doses of cortisone, *Proc. Soc. Exper. Biol. & Med.* **73:** 262–265 (Feb.) 1950.

HORMONE EXCRETION IN LIVER DISEASE AND IN GYNECOMASTIA

J. RUPP, M.D.,* A. CANTAROW, M.D.,** A. E. RAKOFF, M.D.† AND K. E. PASCHKIS, M.D.‡

From the Division of Endocrine and Cancer Research Jefferson Medical College and Hospital, Philadelphia, Pa.

GYNECOMASTIA occurs in 7 to 15 per 100,000 otherwise apparently normal adult men (1, 2). Transient gynecomastia is not uncommon in the new-born (3) and occurs during puberty in about 50 per cent of boys. It has been reported in association with the following diseases: adrenocortical tumors (4, 5, 6), chorioepitheliomas (7), other testicular tumors (8, 9), degeneration of the seminiferous tubules (10, 11), thyrotoxicosis (12), malnutrition (13, 14), paraplegia (15), and liver disease (16, 17, 18). It has been observed also following administration of desoxycorticosterone acetate (19), adrenal cortical extract (20), and testosterone (21, 22).

The question may be raised as to whether or not the development of gynecomastia under these various circumstances is dependent on the operation of a common mechanism. Because of the influence of estrogen upon mammary ductal growth and the frequent occurrence of gynecomastia in men treated with, or otherwise exposed to estrogens, this hormone has been suspected to be involved in the pathogenesis of all forms of gynecomastia. In an attempt to investigate this matter, two groups of patients were studied; 1) subjects with gynecomastia occurring in the absence of other abnormality ("primary" gynecomastia) or in association with some condition other than disease of the liver; and 2) subjects with liver disease, with and without gynecomastia.

There have been few reported studies of hormone excretion in "primary" gynecomastia. Demonstration of the capacity of the liver for inactivation (23, 24, 25) and excretion (26) of estrogen has suggested the possibility that the gynecomastia which has long been recognized as occurring frequently in patients with cirrhosis may be due to an increased concentration of estrogen in the circulation consequent upon inability of the damaged

Received for publication January 18, 1951.

* Dept. of Medicine.

** Dept. of Biochemistry.

† Dept. of Gynecology and Obstetrics.

‡ Dept. of Medicine and of Physiology.

liver to remove it efficiently from the blood stream (27, 28). However, comparatively little factual evidence has been reported in support of this hypothesis.

MATERIALS AND METHODS

In the first group, 25 patients with liver disease were studied, 17 with cirrhosis, 4 with infectious hepatitis, and 4 with extensive metastatic cancer of the liver. The second group comprised 22 patients with gynecomastia without evidence of liver disease, 5 of whom had another disease.

Urinary estrogens were assayed on castrated mice by the vaginal smear method of Allen and Doisy. Gonadotropin assays were performed on infantile mice by the uterine-weight method, using a purified alcohol precipitate of urine. Urinary neutral 17-ketosteroids were determined by the method of Holtorff and Koch (29).

The following values are regarded as normal in adult men according to the experience in our laboratories: estrogen—total, less than 24 m.u.[1]; free, less than 6 m.u.; gonadotropins, 6 to 24 m.u.; 17-ketosteroids, 10–20 milligram equivalents of androsterone per twenty-four hours.

Liver function studies included determination of serum bilirubin, bromsulfalein retention, thymol turbidity, cephalin-cholesterol flocculation, and serum albumin and globulin.

RESULTS

The pertinent findings are summarized in the accompanying tables. Twelve of the 17 patients with cirrhosis excreted abnormally large amounts of estrogen and 9 excreted varying amounts of free estrogen (Table 1). Gynecomastia was present in 5 of the patients exhibiting high urinary estrogen titers, 3 of whom also excreted free estrogens. One patient with gynecomastia (Case 6) had normal urinary estrogen values.

Two of the 4 patients with infectious hepatitis and 2 of the 4 patients with metastatic cancer of the liver excreted abnormally large amounts of estrogen. In 1 hepatitis case and in 2 cancer cases, free urinary estrogens were demonstrable. None had gynecomastia.

17-Ketosteroid excretion was low in all men with liver disease. One young man, 26 years old (Case 18) had normal excretion on a subsequent determination.

Gonadotropin assay yielded a high value in 1 case (No. 23), a 66-year-old male with metastatic liver cancer. In another case (No. 14), with cirrhosis of the liver, the excretion was moderately high.

The 13 patients with "primary" gynecomastia (Table 2) ranged in age

[1] In our laboratories, by the assay method used, 1 mouse unit equals about 5 international units.

TABLE 1. HORMONE EXCRETION IN MALE PATIENTS WITH DISEASE OF THE LIVER

Cases 1–17, Cirrhosis; Cases 18–24, Hepatitis; Cases 22–25, Metastatic carcinoma

Case	Age (yrs.)	Hormone excretion				Liver function						Gynecomastia	Ascites	Remarks
		17-ketosteroids, mg./24 hrs.	Gonadotropins, m.u./24 hrs.	Estrogens, m.u./24 hrs.		Bilirubin, mg./100 ml.	BSP*	Cephalin cholesterol	Thymol turbidity (units)	Alb., Gm./100 ml.	Glob., Gm./100 ml.			
				Free	Total									
1	40	1.5	<6	12 44	44 132	10	60	2	12			Yes	Yes	Autopsy: hemochromatosis; testicular atrophy. Estrogen assays performed 2 weeks apart.
2	45			132	>200	28		4	26	4.92	2.5	Yes	Yes	Needle biopsy: hepatic cirrhosis. Good recovery.
3	26	3.1	<6	< 12	12 200	1.8	41	1	3.2	5.84	1.3	Yes	No	Pulmonary fibrosis; progressive cor pulmonale. Gynecomastia for 3 months prior to death. No autopsy. Assays 3 weeks apart, after development of gynecomastia.
4	56	4.2	<6	132 < 12 12	132 44 >132	0.4 0.7	15	3	8.7	4.46	1.9	Yes	Yes	Esophageal varices. The first two assays were performed 2 weeks apart, and 9 months prior to onset of gynecomastia, at which time third assay was performed.
5	14	0.7	<6		132	3.4	30	4	3.0	4.36	2	Yes	Yes	Juvenile cirrhosis for 4 years; normal secondary sex characteristics. Gynecomastia for 2 years. Assays performed 6 days prior to death. Autopsy: hepatic cirrhosis; testicular atrophy.
6	50	3.8 0.7	<6 <6	12 12	12 24	0.7	0	4	10.2	3.48	3.3	Yes	No	Gynecomastia for 6 months prior to death. Autopsy: hepatic cirrhosis. Hormone assays performed 5½ months apart after the onset of gynecomastia.
7	62	2.9	<6	132	>200	18		4	12	3.8	3.2	No	Yes	Pleural effusion; varices. Autopsy: hepatic cirrhosis.
8	40	3.5		200	132	1.3	0	4	10.6	4.29	2.6	No	No	Autopsy: hepatic cirrhosis. Main manifestation, anemia and varices.
9	64	6.3	27	12	132 64	1.6	46	2	19.4	3.32	4.3	No*	No	Assays two weeks apart. Fair recovery.

10	49	2.0	<6	< 12	64	1.6	5	3	13.4	4.32	3.3	No	Yes	Death following portal caval shunt.
11	37	2.0	<6	< 12	44	2.6	61	4	7	3.39	3.5	No	No	Fair recovery.
12	42	2.2	<6	64 >132	64 >132	20	43	1	3.4	2.46	3.9	No	Yes	Severely ill; fever, pleural effusion. Good recovery.
13	62	3.7	<6	< 12	44	2.3	0		8	5.1	1.9	No	No	Mild disease, good recovery.
14	39	8.8	72	24	24	0.4	11		2.5			No	No	Mild disease, good recovery.
15	46	5.2	<6	< 12	24	8.6	16	4	7	5.48	1.3	No	No	Good recovery.
16	40	3.6	<6	< 12	< 12	0.9	75	3	4.9	4.6	2.4	No	No	Good recovery.
17	62	6.6			12	1.1	0			5.02	2.6	No	No	Biopsy: hepatic cirrhosis. Good recovery.
18	26	5.2 10.1	<6 <6	< 12	12 64	12 6.4	55 40	3 3	4.5 4	5.84	1.9	No	No	Acute hepatitis. Assays performed two weeks apart. At time of second assay, patient was symptomatically better.
19	19	5.8	<6	< 12	12	8.2	38	4	19			No	No	Infectious hepatitis.
20	18		<6	12	132	6.4	15	4	17	4.96	3.2	No	No	Infectious hepatitis.
21	32			12	24	4.2	38	3	16			No	No	Infectious hepatitis.
22	54	9.4	21		64	0.1	0	2	1	4.54	3.3	No	Yes	Embryonal carcinoma of testis with metastases to liver and lung.
23	66	4.2	144	24	24	0.6	0			4.79	2.1	No	Yes	Carcinoma of cecum with metastases to liver.
24	62	1.2	<6	12	44	0.5	0		2			No	Yes	Carcinoma of pancreas with metastases to liver.
25	59	3.2	<6	< 12	12	19.2		3	2	2.69	2.5	No	Yes	Carcinoma of ampulla of Vater with metastases to liver.

* Thirty minutes after injection of 2.5 mg.

TABLE 2. PUBERTAL GYNECOMASTIA

Case	Age (yrs.)	Hormone excretion				Liver function				Remarks
		17-Keto-steroids, mg./24 hrs.	Gonado-tropins, m.u./24 hrs.	Estrogens, m.u./24 hrs.		Bili-rubin, mg./100 ml.	BSP*	Cephalin chol-esterol	Thymol turbid-ity (units)	
				Free	Total					
1	7	1.8	<6		24					Developed gynecomastia at age 7. First sign of puberty.
2	11	4.4 11	<6	<12 <12	24 200 12	0.4	0		0.8	Gynecomastia at puberty. First two assays performed 2 months apart; third assay performed 4 months after second.
3	12	3	<6		<12					Gynecomastia at puberty; assay at time of puberty.
4	14	5.6	18	12 12 <12 <12 132	>200 44 44 24 200	0.2 0.7	0 0	 1	3 2	Gynecomastia at puberty. First 3 assays performed over a 5-month period; 5th assay performed 2 years after first. Biliary excretion of BSP impaired.
5	14	4	<6	<12	<12					Gynecomastia at puberty; assay at time of onset of gynecomastia.
6	15	8.3			<12					Gynecomastia at puberty; assay at time of onset of gynecomastia.
7	15	9.9	<6		12					Gynecomastia since puberty at age 11.
8	16	8.6	<6	<12	44					Gynecomastia since puberty at age 12; Sperm count normal.
9	17	12	<6	<12	64	0.2	0		1.4	Gynecomastia since puberty at age 13.
10	19	13.2 10	84 21 76		24 24					Normal sperm count. Gynecomastia at puberty, at age 10. Married; one child. Assays performed 2 weeks apart.
11	21	8.2	<6	12	24		0		2.8	Gynecomastia since onset of puberty at age 11.
12	22	4	<6	12	24					Gynecomastia since puberty at age 13.
13	23	12.8	<6	<12	44 132	0.7	0			Gynecomastia since puberty at age 14. Normal sperm count. Assays performed 6 months apart.

* Thirty minutes after injection of 2.5 mg.

TABLE 3. POSTPUBERTAL GYNECOMASTIA ASSOCIATED WITH VARIOUS DISEASES

Case	Age (yrs.)	Hormone excretion				Liver function				Remarks
		17-Ketosteroids, mg./24 hrs.	Gonadotropins, m.u./24 hrs.	Estrogens, m.u./24 hrs.		Bilirubin, mg./100 ml.	BSP*	Cephalin cholesterol	Thymol turbidity (units)	
				Free	Total					
1	19	13.4	<6	12	44					Hemophilia. Gynecomastia developed while patient was recovering from hemarthros.
2	25			12	24	0.5			2.5	Unilateral gynecomastia; normal semen analysis. Breast tissue disappeared spontaneously in 6 months.
3	45	6.5	<6	>200 12 <12	132 12 12	0.7	0		1.4	Gynecomastia developed at age of 45. First two assays were performed 3 weeks apart, 3rd assay 3 months later. Gynecomastia persisted during observation.
4	28	11.1	<6	<12	12	0.7	0	3	7	Inactive acromegaly. Unilateral gynecomastia developed at age 28.
5	62	2.1 3.9	<6 <6		24	0.3	0	2.1	1	Gynecomastia developed at age 62. A small fibroma of the testis was present. Gynecomastia began to disappear prior to removal of mass.
6	42	12.8 12 5.6 11.1	15 88 129 162 120	 0 64 12 24	200 44 200 64 24	0.4 0.9 0.6 0.5	0 0 0	6.2	0	Hyperthyroidism. Recurring unilateral gynecomastia. At time of each recurrence the estrogen excretion was increased. Upon control of hyperthyroidism gynecomastia disappeared. Then gonadotropin secretion increased to high levels. Time intervals between assays: 2nd, 3 mo.; 3rd, 7 mo.; 4th, 14 mo.; 5th, 27 mo.

* Thirty minutes after injection of 2.5 mg.

from 7 to 23 years; gynecomastia developed during puberty, and in some cases had persisted for several years after pubertal development was completed. None had any subjective complaints or objective abnormal findings other than gynecomastia. Liver function studies performed in 6 of these cases yielded normal findings. Case 1 is remarkable in that gynecomastia preceded all other signs of pubertal development; biopsy revealed the typical findings of gynecomastia with duct proliferation.

Estrogen excretion was abnormally high in 2 of 6 cases observed during their pubertal development, and in 3 of 7 cases in which gynecomastia, appearing during puberty, persisted; semen analysis in some of the latter cases yielded normal results. In 1 case in this group, in whom there was

no clinical evidence of liver disease and the usual liver function tests gave normal results, delayed excretion of bromsulfalein in the bile was found in the presence of normal clearance from the blood.[2]

In Table 3, are listed 6 cases in whom gynecomastia developed after puberty. One (Case 4) was an acromegalic, apparently in an inactive phase at the time of appearance of gynecomastia. His hormone excretion values were within normal limits. Gynecomastia in Case 6, a patient with severe thyrotoxicosis, was associated with high estrogen excretion. The size of the breast tissue masses fluctuated considerably, at times becoming almost impalpable. The urinary estrogen values were highest at the time of maximal breast development. When the thyrotoxicosis was controlled by treatment with I^{131}, the gynecomastia disappeared. The gonadotropin (FSH) excretion gradually increased, suggesting testicular failure.

TABLE 4. GYNECOMASTIA ASSOCIATED WITH TESTICULAR FAILURE

Case	Age (yrs.)	Hormone excretion				Liver function				Remarks
		17-Ketosteroids, mg./24 hrs.	Gonadotropins, m.u./24 hrs.	Estrogens, m.u./24 hrs.		Bilirubin, mg./100 ml.	BSP*	Cephalin cholesterol	Thymol turbidity (units)	
				Free	Total					
1	72	7.4	117 135		<12	0.3		1	1.4	Gynecomastia developed at age 72 and lasted 7 months.
2	29	8.8	144 138	<12	<12					Eunuchoid. Prepubertal testicular injury. Could produce only small amount of seminal fluid, no sperm.
3	54	5.6	150	<12	44	0.7	0	1	1	Gynecomastia for 20 years; developed following bilateral hernia repair. Very small testes. Normal secondary sex characteristics.

* Thirty minutes after injection of 2.5 mg.

Four patients had gynecomastia, either as the only detectable abnormality, or associated with probably unrelated disorders, such as hemophilia (Case 1), and a small fibroma of the testis (Case 5). Two of these had high urinary estrogen values.

Three patients listed in Table 4 had evidence of testicular failure, with high FSH excretion. One of these excreted excessive amounts of estrogen.

DISCUSSION

Hormone excretion in liver disease: The urinary neutral 17-ketosteroid values were low in almost all of the cases of liver disease; this finding is in agreement with previous observations by others (17, 27, 28). In the

[2] We are indebted to Dr. Chas. W. Wirts for these studies.

majority of cases the values were much lower than those found in castrates and eunuchoids, suggesting either decreased adrenocortical production of 17-ketosteroid precursors or a disturbance in their intermediate metabolism, in which the liver is known to play an important role (30, 31).

The observation that the urinary 17-ketosteroids increase promptly in cirrhotics after administration of testosterone (32) suggests that the low values encountered in cirrhotics are a reflection of diminished adrenocortical production.

FSH excretion was moderately elevated in 1 cirrhotic, aged 39 (Table 1), and high in 1 man, aged 66, with extensive metastatic cancer of the liver (Table 1, Case 23) and in 3 patients with testicular failure (Table 4, Cases 1, 2, 3). There is no indication in this series that FSH excretion is directly influenced by liver disease.

Estrogens: The role of the liver in estrogen metabolism has been reviewed by us recently (33). Three types of activity in this connection have been demonstrated: 1) excretion in the bile, 2) transformation to compounds of lesser biologic activity and destruction of the steroid molecule, and 3) conjugation. Only a few studies of estrogen excretion in patients with liver disease have been reported (27, 28). Our findings of increased urinary estrogen values in 12 of 17 cases of cirrhosis, 2 of 4 cases of hepatitis and 2 of 4 cases of metastatic cancer of the liver indicate that increased estrogen excretion occurs frequently, although not constantly, in liver disease. It is possible that the incidence of disturbed estrogen metabolism would be found to be higher if assays were performed more frequently. Studies of the removal of exogenous estrogen from the blood. (34, 35) appear to indicate a higher incidence of disturbance of estrogen metabolism than does study of urinary excretion of products of endogenous estrogen metabolism. The presence of free estrogen in the urine in 6 of 10 cirrhotics showing an increase in total urinary estrogen, also previously reported by Glass *et al.* (27), suggests a disturbance in the conjugating mechanism, which appears less sensitive than the other mechanisms. The clinical observations do not permit differentiation between impaired biliary excretion and degradation of the steroid molecule. Animal experiments suggest that the former is much more sensitive to impairment of liver function than the latter.

No correlation was found between disturbance in estrogen metabolism and the degree of liver functional impairment, as gauged by various tests of liver function, or by the severity of clinical manifestations. An observation made in 1 case of gynecomastia (Table 2, Case 4), may be pertinent in this connection. This patient had so-called idiopathic gynecomastia, with high urinary estrogen values; there was no clinical indication of liver disease and the usual liver function tests yielded normal results. However,

the biliary excretion of bromsulfalein (BSP) was delayed in the presence of normal clearance from the blood (36). This case demonstrates the sensitivity of the mechanisms involved in estrogen metabolism. Case 18 (Table 1) is also instructive. In this instance, high urinary estrogen titers occurred only at a time when other functions of the liver, as evidenced by various tests, were improving, whereas estrogen excretion had been normal at the height of the liver functional impairment. This indicates a highly specialized functional disturbance of estrogen metabolism.

Gynecomastia: Thirteen of the 28 cases of gynecomastia (Tables 1–4) showed increased urinary estrogen excretion. Analysis of the clinical findings and hormonal patterns in the patients with high estrogen titers as compared with those with normal estrogen excretion failed to reveal any consistent correlation among these data. The findings in subjects with liver disease, summarized in Table 5, indicate that gynecomastia may be absent in the presence of high urinary estrogen titers and, conversely, may

TABLE 5. URINARY ESTROGENS IN HEPATIC DISEASE WITH AND WITHOUT GYNECOMASTIA

	Gynecomastia Urinary estrogen			*No Gynecomastia* Urinary estrogen		
	High	Normal	Free	High	Normal	Free
Cirrhosis	5*	1	3	7	4	6
Hepatitis				2	2	1
Cancer metastases				2	2	2

* Figures indicate number of cases in each group.

be present in association with normal urinary estrogen values. Excretion of free estrogens occurred in liver disease both with and without gynecomastia. Differences in sensitivity of the target tissue, namely the breast anlage, might be considered to be a determining factor in this connection, possibly conditioned by the nutritional status, but the data lend little support to such an assumption. The nutritional condition of certain of the patients with severe cirrhosis was certainly more seriously impaired than that of the patients with hepatitis; in the latter, gynecomastia was not present. An intrinsic difference in tissue response, rather than one caused by nutritional factors, might explain absence of gynecomastia in the presence of high estrogen excretion. The fact that gynecomastia occurs only in some, but not in all men industrially exposed to estrogen, is in keeping with such an assumption.

On the other hand, 53 per cent of the patients with gynecomastia had normal urinary estrogens at the time of study. This might suggest that

factors other than excess of circulating estrogen may be involved in the production of gynecomastia. However, it is conceivable that an excessive estrogenic stimulus at some time other than that of the hormone assay might have stimulated development of the breast, which, once established, would have been maintained by smaller amounts of estrogen. This would be in keeping with other observations which indicate that amounts of a hormone necessary to initiate an effect are usually larger than the required "maintenance dose." Actually, gynecomastia occurring incident to the therapeutic use of large doses of estrogens, sometimes persists after cessation of therapy, whereas in other individuals it regresses. The data presented throw no additional light upon these possibilities. More detailed study of individual cases may contribute additional information.

SUMMARY

Hormone excretion was studied in 25 patients with liver disease, 6 of whom had gynecomastia, and in 22 patients with gynecomastia without liver disease.

In patients with liver disease, 17-ketosteroid excretion was low. Gonadotropin excretion showed no significant changes. Estrogen excretion was high in 16 of the 25 cases, with an increase in the free moiety in 12. Presence of gynecomastia in liver disease was not correlated to the presence of high urinary estrogen excretion, nor to the excretion of nonesterified estrogens.

In the 22 cases of gynecomastia without demonstrable liver disease, increased urinary estrogen values were recorded in 9 patients, with an increased free moiety in 7.

These findings emphasize the role estrogens play in gynecomastia. The problem presented by the cases with normal estrogen excretion is discussed.

REFERENCES

1. Webster, G. V.: Gynecomastia in the navy, *Mil. Surgeon* **95:** 375–379, 1944.
2. Spankus, W. H., and Grant, R. S.: Gynecomastia, *J. Clin. Endocrinol.* **7:** 586–601, 1947.
3. Emmens, C. W., and Parkes, A. J.: Effects of exogenous estrogens in male mammals, *in* Vitamins and Hormones, New York, Academic Press, 1947, vol. 5, p. 235.
4. Wilkins, L.: A feminizing adrenal tumor causing gynecomastia in a boy of five years contrasted with a virilizing tumor in a five-year-old girl, *J. Clin. Endocrinol.* **8:** 111–132, 1948.
5. Staffieri, J. J.; Cames, O., and Cid, J. M.: Corticoadrenal tumor with hypoglycemic syndrome, goiter, gynecomastia and hepatosplenomegaly, *J. Clin. Endocrinol.* **9:** 255–267, 1949.
6. Chambers, W. L.: Adrenal cortical carcinoma in a male with excess gonadotropin in the urine, *J. Clin. Endocrinol.* **9:** 451–456, 1949.
7. Clinico-Pathologic Conference: Mediastinal tumor with gynecomastia and superior vena caval obstruction, *Am. J. Med.* **6:** 247–258, 1949.

8. Östergaard, E.: Feminizing tumor of the testis, presumably aberrant adrenocortical tumor, *J. Clin. Endocrinol.* **7**: 438–445, 1947.
9. Lewis, L. G., and Stockard, C. S.: Feminizing testis tumors, *J. Urol.* **64**: 518–523, 1950.
10. Klinefelter, H. F., Jr.; Reifenstein, E. C., Jr., and Albright, F.: Syndrome characterized by gynecomastia, aspermatogenesis without a-leydigism, and increased excretion of follicle-stimulating hormone, *J. Clin. Endocrinol.* **2**: 615–627, 1942.
11. Heller, C., and Nelson, W. O.: Hyalinization of the seminiferous tubules associated with normal or failing Leydig-cell functions, *J. Clin. Endocrinol.* **5**: 1–13, 1945.
12. Starr, P.: Gynecomastia during hyperthyroidism, *J.A.M.A.* **104**: 1988–1990, 1935.
13. Klatskin, G.; Salter, W. T., and Humm, F. D.: Gynecomastia due to malnutrition; clinical studies, *Am. J. M. Sc.* **213**: 19–30, 1947.
14. Jacobs, E. C.: Gynecomastia following severe starvation, *Ann. Int. Med.* **28**: 792–797, 1948.
15. Cooper, I. S., and Hoen, T. I.: Gynecomastia in paraplegic males, *J. Clin. Endocrinol.* **9**: 457–461, 1949.
16. Edmonson, H. A.; Glass, S. J., and Soll, S. N.: Gynecomastia associated with cirrhosis of the liver, *Proc. Soc. Exper. Biol. & Med.* **42**: 97–99, 1939.
17. Lloyd, C. W., and Williams, R. H.: Endocrine changes associated with Laennec's cirrhosis, *Am. J. Med.* **4**: 315–327, 1947.
18. Klatskin, G., and Rappaport, E.: Gynecomastia due to infectious hepatitis of the homologous serum type, *Am. J. M. Sci.* **214**: 121–127, 1947.
19. Lawrence, R. D.: Gynecomastia produced by desoxycorticosterone acetate, *Brit. M. J.* **1**: 12, 1943.
20. Edwards, R. A.; Shimkin, M. R., and Shaver, J. S.: Hypertrophy of the breast due to injections of adrenal-cortical extract in a man with Addison's disease, *J. A.M.A.* **111**: 412–414, 1938.
21. McCullagh, E. P., and Rossmiller, H. R.: Methyltestosterone. I. Androgenic effects and the production of gynecomastia and oligospermia, *J. Clin. Endocrinol.* **1**: 496–502, 1941.
22. Howard, J. E., and Scott, W. W.: *in* Textbook of Endocrinology, edited by R. H. Williams, Philadelphia, W. B. Saunders Company, 1950, p. 331.
23. Zondek, B.: Ueber das Schicksal des Follikelhormones (Follikulin) in Organismus, *Skandinav. Arch. f. Physiol.* **70**: 133, 1934.
24. Heller, C. G., and Heller, E. J.: Metabolism of estrogens: effect of pregnancy upon metabolism *in vitro* of estrone, estradiol, and estriol, *Endocrinology* **32**: 64–68, 1943.
25. DeMeio, R. H.; Rakoff, A. E.; Cantarow, A., and Paschkis, K. E.: Mechanism of inactivation of α-estradiol by rat liver "in vitro," *Endocrinology* **43**: 97–104, 1948.
26. Cantarow, A.; Rakoff, A. E.; Paschkis, K. E.; Hansen, L. P., and Walking, A. A.: Excretion of estrogen in bile, *Endocrinology* **31**: 515–519, 1942.
27. Glass, S. J.; Edmonson, H. A., and Soll, S. N.: Sex hormone changes associated with liver disease, *Endocrinology* **27**: 749–752, 1940.
28. Gilder, H., and Hoagland, C. L.: Urinary excretion of estrogens and 17-ketosteroids in young, adult males with infectious hepatitis, *Proc. Soc. Exper. Biol. & Med.* **61**: 62–65, 1946.
29. Holtorff, A. F., and Koch, F. C.: The colorimetric estimation of 17-ketosteroids and their application to urine extracts, *J. Biol. Chem.* **135**: 377–392, 1940.

30. PASCHKIS, K. E.; CANTAROW, A.; RAKOFF, A. E.; HANSEN, L. P., and WALKING, A. A.: Excretion in the dog of androgens and estrogens in the bile following the injection of androgens, *Proc. Soc. Exper. Biol. & Med.* **55**: 127–130, 1944.
31. SAMUELS, L. T.; SWEAT, M. L.; LEVENDAHL, B. H.; POTTNER, M. M., and HELMREICH, M. L.: Metabolism of testosterone by livers of different species of animals, *J. Biol. Chem.* **183**: 231–239, 1950.
32. WILLIAMS, T. L.; CANTAROW, A.; PASCHKIS, K. E., and HAVENS, JR., W. P.; Urinary 17-ketosteroids in chronic liver disease, *Endocrinology* (in press).
33. PASCHKIS, K. E., and RAKOFF, A. E.: Some aspects of the physiology of estrogenic hormones, *in* Recent Progress in Hormone Research, New York, Academic Press, 1950, vol. 5.
34. RAKOFF, A. E.; CANTAROW, A.; PASCHKIS, K. E., and HANSEN, L. P.: The clearance of injected estrogens from the blood of normal humans and those with liver disease, *Fed. Proc.* **3**: 1944.
35. ZONDEK, B., and BLACK, R.: Estrone clearance in infectious hepatitis, *J. Clin. Endocrinol.* **7**: 519–529, 1947.
36. WIRTS, C. W., and CANTAROW, A.: Study of excretion of bromsulphthalein in bile, *Am. J. Digest. Dis.* **9**: 101–106, 1942.

STUDIES CONCERNING THE MECHANISM OF WATER RETENTION IN ADDISON'S DISEASE AND IN HYPOPITUITARISM

ALEXANDER SLESSOR, M.B., F.R.F.P.S.*

From the Peter Bent Brigham Hospital, Boston, Massachusetts, and the University Medical Clinic, Stobhill Hospital, Glasgow, Scotland

A DISTURBANCE of renal function has been known to be present in adrenal insufficiency since 1916, when Marshall and Davis (1) reported that adrenalectomized cats have a markedly depressed kidney function before the phase of terminal failure. In 1931 Rowntree and Snell (2) made similar observations in Addison's disease. They reported that the dilution test disclosed "a decided lag in the onset of diuresis and in the total secretion of water." This observation—failure of prompt diuresis—was later taken up by Robinson, Power and Kepler (3) and made the basis of a useful screening test for Addison's disease.

Many studies have been made to determine the mechanism of the faulty water excretion in adrenal insufficiency and these have been reviewed by Gaunt (4, 5) and Levy, Power and Kepler (6). The phenomenon has not appeared to be a simple one. Various combinations of the following factors have been considered as responsible: stasis of water in the bowel, abnormal distribution of water in the body, a fault at the renal level, and increased activity of the posterior pituitary gland. Although defective absorption from the gut is a well known finding in adrenal insufficiency, it does not appear to be an essential factor in the faulty water diuresis; for isotonic fluids given intravenously (6, 7) or intraperitoneally (8, 9) still fail to be excreted promptly. Observations are conflicting as to whether the delayed water excretion in adrenal insufficiency is due primarily to an abnormal fluid distribution in the tissues. In adrenalectomized rats Gaunt (9) has presented indirect evidence suggesting that ingested water passes principally into the intracellular phase; on the other hand, Shipley's studies indicate that it is mainly extracellular (10). Both are agreed that the blood volume is not increased following water ingestion and this would indicate that the water is not available for ready excretion by the kidneys in adrenalectomized animals. No clinical studies on this aspect of the problem have been reported up to the present time.

Received for publication January 10, 1951.

* Commonwealth Fund Travelling Fellow 1948–49. Present address: Materia Medica Department, The University of Glasgow, Glasgow, W.2., Scotland.

At the renal level, defective excretion of water may occur theoretically from diminished filtration or increased reabsorption by the tubules or by a combination of both. There is ample evidence that glomerular filtration is diminished in primary and secondary adrenal failure, both in animals (11–15) and in man (16–19). Adequate treatment of clinical adrenal insufficiency with DCA restores the filtration rate to normal or nearly normal levels (16, 19), yet the prompt diuretic response to water is still lacking (6.) Salt-maintained adrenalectomized rats show essentially normal filtration rates (15, 20, 21) but when such animals are left untreated the urinary volume falls to a greater extent than can be accounted for by the diminishing glomerular filtration (15). On the basis of their findings, Lotspeich (20) and Boss, Birnie and Gaunt (15) consider accelerated tubular reabsorption to be an important factor in the failure of diuresis. This recent work confirms observations made by Gersh and Grollman (12) who found evidence for increased tubular reabsorption in adrenalectomized rats in which water reabsorption was measured by the degree of in vivo staining of the tubular cells by ferricyanide.

As originally shown by Starling and Verney (22), the posterior pituitary hormone is concerned with the reabsorption of water by the renal tubules. In 1939 Martin *et al.* (23) observed increased quantities of antidiuretic substance in the urine of untreated adrenalectomized cats. Recently Birnie *et al.* (25) have found increased quantities of antidiuretic substance of pituitary origin in the fasting serum of adrenalectomized rats. In 1946 our attention was drawn to the possibility that increased antidiuretic activity might be playing a part. In the plasma of 2 patients with anterior hypopituitarism, both of whom showed marked water retention, were found quantities of antidiuretic material which substantially diminished a saline-induced diuresis in rabbits (26).

In summary, it would appear from animal studies that failure of diuresis results essentially from a disturbance of kidney function. Diminished filtration has been established as a factor in untreated adrenalectomized animals but more important is increased tubular reabsorption brought about by an antidiuretic substance in the blood, probably of neurohypophyseal origin. In human studies, diminished filtration rate from a subnormal renal plasma flow is of some importance, but there still remains for examination the possible role of increased tubular reabsorption from the action of an antidiuretic agent.

The purpose of the present study was to determine if there is any disturbance of the posterior pituitary mechanism for the control of water excretion in patients with adrenal insufficiency. Since Verney (27) has shown that the physiologic control of the neurohypophysis is brought about by the level of osmotically active ions in the plasma, the dilution of the

serum electrolytes following a standard dose of water has been measured. Observations were also made to determine whether a delayed diuresis occurred in patients who failed to show a normal or prompt response to water ingestion. Assays for antidiuretic substance have been made of 1) the serum of normal subjects and of patients with Addison's disease after

TABLE 1. SUMMARY OF CASES OF ADRENAL INSUFFICIENCY, PRIMARY AND SECONDARY

Patient	Age (yrs.)	Sex	Probable etiology	Duration of disease (yrs.)	Salt deficiency	Carbohydrate deficiency	Urinary 17-ketosteroids, mg. per 24 hrs.	Treatment prior to water test
M.Ht.	30	F	Atrophy	1½	+	±	3.2	NaCl, 3 Gm. daily; DCA in oil 2.5 mg. twice weekly; cortical extract when necessary. For 7 days before test, no therapy save 3 Gm. NaCl daily. B.P. day of test, 90/66.
C.S.	44	F	Atrophy	20	+	++	0.7	No treatment for 3 days prior to water test. Before admission taking supplementary salt with food. B.P. 90/58.
W.D.	55	M	Adrenal hemorrhage	15	++	+	2.2	Prior to admission, 3 cc. of cortical extract daily and supplementary salt. No extract for 2 days before test but taking 6 Gm. NaCl daily. B.P. on day of test, 100/68.
R.W.	66	M	Atrophy	10	+	±	3.9	Taking 1.5 mg. DCA at time of water test. No NaCl. B.P. 100/68.
H.W.	33	M	Atrophy	6	++	+	3.2	DCA pellets (500 mg.) implanted 14 months before. Prior to admission was taking 5 cc. of cortical extract and 1.5 mg. DCA in oil. No therapy for 3 days prior to test.
J.C.	54	M	Atrophy	15	+	±	2.1	DCA pellets (250 mg.) implanted 10 months before. No supplementary treatment outside, or before test. B.P. 106/70.
G.T.	42	M	Chromophobe adenoma	13	±	±	1.1	Pituitary radiation, 13 years before. Present therapy, thyroid ½ gr. daily. B.P. at time of test, 102/68.
M.R.	48	M	Chromophobe adenoma	4½	±	±	2.4	Thyroid, ½ gr. daily; no other treatment. B.P. 112/60. Radiation, 4,500 r, to pit. tumor 3 yrs. previously.
S.D.	67	M	Chromophobe adenoma	17	±	−	2.7	Pituitary radiation, 4,500 r, 12 yrs. before, and 4500 r 3 months before. No other therapy. B.P. 200/110.
H.L.*	49	M	Epidermoid	20	±	+	2.4	Testosterone, 600 mg. implanted 8 months before. Taking thyroid, 1 gr. daily. BMR −39%. B.P. on morning of test, 110/70.
M.Hm.*	42	F	Postpartum necrosis of anterior lobe	9	±	+	3.6	Thyroid, 1½ gr. daily; NaCl, 6 Gm. daily.

* Cases studied in the University Medical Unit, Stobhill Hospital, Glasgow.

a period of fourteen hours without water; and 2) urine voided under conditions of mild dehydration and hydration as outlined below.

MATERIAL, PROCEDURE AND METHODS

Clinical material

The clinical material used in this investigation consisted of 6 patients with Addison's disease and 5 patients with hypopituitarism from various causes. None of the patients was in poor clinical condition, but most of the Addisonians were in the hospital for reimplantation of pellets and 1 of these required maintenance doses of DCA. Some clinical data concerning the patients studied, together with the maintenance therapy, are presented in Table 1. The normal subjects consisted of healthy males who were working in the laboratory.

Procedure

The water test of Robinson, Power and Kepler (3) was used, not only to demonstrate water retention but as a basis for a more complete examination of the phenomenon. In order to guard against a spontaneously reduced fluid intake which would stimulate posterior pituitary activity, each patient was required to ingest 2,000 to 3,000 ml. of fluid per twenty-four hours for a minimum of two days before the water test. On the evening before the test no food or fluids were allowed after 6:30 P.M.; at 10:30 P.M. urine was voided and discarded. All urine passed from 10:30 P.M. to 7:30 A.M., the patient voiding at the latter time, was collected and labelled "night urine." At 8:30 A.M. the bladder was again emptied and the specimen labelled "fasting urine." Water was then given, 20 ml. per kilogram of body weight, thirty to forty-five minutes being taken for its ingestion. At hourly intervals from 8:30 A.M. to 12:30 P.M. the bladder was emptied and the entire volume at each voiding saved. The following determinations were made on the urinary specimens: volume, specific gravity, chlorides, urea (night specimen only). Assays were made of the antidiuretic activity of the night urine, and of the urine passed during the four hours of the water test.

Blood samples were taken just before the water was ingested and hourly throughout the four hours of the test. On all these spceimens the hematocrit, total serum proteins, and total serum base were determined. On the fasting blood specimen, plasma urea and chlorides were estimated for the calculation of value A (procedure II of water test (3)).

$$A = \frac{\text{Urinary urea nitrogen}}{\text{Plasma urea nitrogen}} \times \frac{\text{plasma Cl}}{\text{urinary Cl}} \times \frac{\text{max. hourly vol. in ml.}}{\text{night vol. in ml.}}$$

Estimations of the urinary chloride and urea nitrogen were made on the night urine.

When a diuresis did not occur during the four hours of the water test, observations were made to determine if a diuresis would occur in the next twenty hours, *i.e.*, until 8:30 A.M. the next morning. At approximately two-hourly intervals, therefore, the patient was asked to void, volumes and specific gravities were noted, and the results charted as urine flow in ml. per hour. On three of the occasions when a late diuresis was noted, the antidiuretic activity of the urine voided at the time of the diuresis was determined and compared with that of the urine passed in the four hours of the water test.

In 4 of the patients being tested and in 3 other Addisonian patients not undergoing the water test, estimations of the antidiuretic activity of the serum after a period of fourteen hours without water were determined and compared with the activity of serum from normal subjects taken under identical conditions.

Methods

Urinary and plasma chlorides were estimated by the method of Schales and Schales (28, 29); urinary urea nitrogen by Van Slyke's hypobromite method (30); plasma urea by the method of Archibald (31); total serum proteins by the falling-drop method (32); and total serum base by the conductivity bridge method of Sunderman (33). The hematocrit reading was taken after spinning the blood at 3,000 r.p.m. for thirty minutes.

Antidiuretic assays

Urine: For the extraction of urine for antidiuretic principle, use was made of the ultrafiltration technique devised by Gorbman (34). By this method the urine, made slightly alkaline, is passed through a 2 per cent collodion membrane under a pressure of 10–30 lbs. When the filtration is finished, the membrane is dissolved in alcohol ether and spun, the supernatant fluid containing the collodion being poured off. After three washings with alcohol ether and a final one with anhydrous ether, the material remaining is dried by a gentle current of air. It can now be stored in a refrigerator until immediately before the assay is to be made, when it is dissolved in saline so that each ml. of solution contains the amount of material passed in a 20-minute secretion of urine. For the assays, rats were used and the dose given intraperitoneally was 1 ml. per 100 grams of weight.

The method of assay employed was that introduced by Ham and Landis in 1942 (35). In a preliminary trial of several methods (36, 37) this was found to give the most consistent response to graded doses of pitressin (P.D.). An attempt was also made to use rats in which diabetes insipidus had been produced by the removal of the posterior pituitary lobe.[1] Such animals

[1] Dr. Roy Greep of the Department of Dental Anatomy, Harvard Dental School, kindly performed the neurohypophysectomies for this study.

exhibited the diuretic phenomenon for short periods only (10–20 days) and were therefore found unsatisfactory for assay purposes. Following the technique of Ham and Landis, 18 male rats of the Hisaw strain, on a constant weighed diet, were fasted overnight but had free access to a liberal water supply. The rats were studied in groups of 3 animals and so arranged that the total weight of each group was approximately 600 grams. On the morning of the test at 9 A.M. each rat was given by stomach tube an initial priming dose of 0.2 per cent NaCl solution, 2.5 ml. being given for each 100 grams of rat weight. Two hours later the test dose, 5 ml. per 100 Gm., was administered. Immediately prior to the test dose the rats were injected intraperitoneally with normal saline (controls) or with the urine extracts obtained from (a) night urine or (b) urine passed during the four hours of the water test or (c) urine passed at the time of diuresis when this was delayed. In each assay, 2 groups of 3 animals acted as controls, 2 groups were given extract (b) and 2 groups were given either (a) or (c). Cumulative urine volumes were measured for each group at 15-minute intervals for three hours and the volumes calculated as ml. per 100 grams of rat weight. In each assay the mean curve of the two control groups and the mean curve of each set of two test groups were charted on squared paper (1 ml. marked off 1 inch on the ordinate and 30 min. marked off 1 inch on the abscissa (Fig. 1)) and the area under each curve measured. In

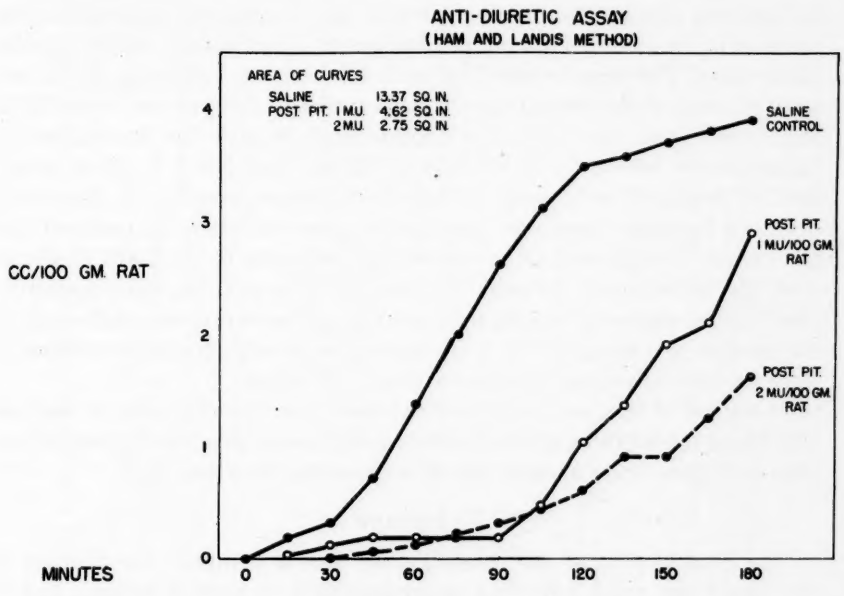


FIGURE 1

the series of 23 assays, the difference between the areas of the two control curves of any one test was no greater than 1.1 sq. inch, except on two occasions when the differences were 3.22 and 2.48 sq. inches respectively. In these cases the lower of the two curves was taken as the control. Solutions showing antidiuretic activity gave a curve of smaller area than the control and the degree of antidiuretic activity was measured by the difference between the control and test curve areas. The degree of antidiuretic activity was arbitrarily expressed (35) as follows: when the difference between the mean test curve and the mean control curve (or the lower of two controls in those giving high values) was

less than 2 sq. inches = no antidiuretic activity
2–4 sq. inches = slight antidiuretic activity
4–8 sq. inches = moderate antiduretic activity
greater than 8 sq. inches = marked antidiuretic activity

Serum: Attempts to measure the antidiuretic activity of serum by the Ham and Landis method failed. The method was not sufficiently sensitive. Drs. Birnie and Gaunt kindly supplied details of their method before publication while the present study was under way. This method (24) proved to be very sensitive and all the assays on serum have been carried out using this technique slightly modified. In this method, as employed by us, fasting male rats are hyperhydrated (stomach tube) by giving hypotonic NaCl 0.2 per cent solution (water alone gives a high incidence of hemoglobinuria in the rats) in a dose of 4.5 ml. per 100 grams of rat weight, every hour for three doses. The urine secreted by each rat from the beginning of the test until the end of the second hour is measured and this pre-test excretion is subtracted from the total dose administered to give the load of water. Immediately before the third dose of 0.2 per cent NaCl, 1 ml. of serum per 100 grams of body weight is injected intraperitoneally. At 15-minute intervals for three hours the cumulative urine excretion is measured and the volume is expressed as the percentage excretion of the load. As Birnie *et al.* (24) have found, the value at ninety minutes gave the most consistent results. The degree of antidiuretic activity of the serum was indicated by the smaller percentage of the load excreted at ninety minutes as compared with the control animals injected with normal saline.

In our use of this method, 2 to 4 rats were used for the assay of each serum being tested and a similar number acted as controls. Each group of rats was used three times at intervals of not less than fourteen days.

RESULTS

The general plan of the investigation was to compare the changes in the blood and urine following water ingestion in normal persons and in patients with adrenal cortical insufficiency.

TABLE 2. BLOOD AND URINE CHANGES FOLLOWING WATER INGESTION (20 ML. PER KG.) IN NORMAL PERSONS AND IN PATIENTS WITH ADDISON'S DISEASE AND HYPOPITUITARISM

	Urinary volumes in ml.						Specific gravity of urine						Value "A"	% excrn. of water dose	Urinary chlorides, mEq./hr.						Hematocrit					Total serum base, mEq./L				
	N.U.*	F.U.†	1	2	3	4	N.U.	F.U.	1	2	3	4			N.U.	F.U.	1	2	3	4	F.	1	2	3	4	F.	1	2	3	4
			(hrs. after water)						(hrs. after water)								(hrs. after water)						(hrs. after water)								
Normal subjects																														
A.S.—1	490	29	245	735	330	78	1021	1026	1005	1002	1002	1009	78	85	6.11	6.46	8.87	4.54	5.44	5.68	49.5	49.5	51	50	50	156	151	154	154	156
A.S.—2	290	29	152	710	192	40	1027	1029	1010	1003	1006	1018	124	65	5.56	6.93	10.75	8.60	5.82	6.06	50.5	50.5	50	50	50	153	149	151	152	152
F.G.	945	34	57	630	410	126	1016	1026	1020	1003	1003	1008	32	76	9.46						49	50	51	50	49	149	146	145	149	150
L.B.	485	81	215	730	290	54	1026	1023	1010	1003	1005	1015	202	78	7.70	19.30	21.9	13.9	9.6	6.7	48	48	47.5	47	46.5	150	141	147	153	146
Addison's disease																														
M.Ht.	228	29	52	160	148	92	1026	1026	1020	1006	1007	1009	13	45	3.87	6.15	8.34	6.78	7.18	5.57	35	36	36.5	36.5	34	145	142	140	144	143
C.S.	290	26	—	—	56	—	1012	1018	—	—	1017	—	4	15	1.63	2.9	—	—	6.16	—	37	37	35	34	34	143	140	137	138	140
W.D.	388	27	50	88	97	93	1019	1021	1018	1012	1011	1011	5	29	5.61	—	—	—	—	—	45	45	41	41	41	147	145	141	137	137
R.W.	870	105	—	7	45	49	1011	1012	—	—	1015	1015	2	17	6.67	—	—	—	—	—	47	46	44	43	45	142	139	138	137	138
H.W.	235	36	36	38	29	21	1024	1023	1024	1017	1019	1021	18	8	5.66	3.21	5.9	4.2	2.8	2.0	42	—	40	40	39	150	145	142	141	143
J.C.	155	53	34	30	27	24	1026	1023	1022	1018	1017	1020	7	11	2.75	8.11	4.68	3.27	2.48	2.76	41	41	41	40	40	140	138	134	136	135
Hypopituitarism																														
G.T.	440	—	28	35	35	20	1014	—	1020	1020	1019	1019	3	9	2.35	2.49	3.12	3.01	4.83	2.22	41	41	41	40	40	143	142	137	139	140
M.R.	370	34	48	48	39	28	1018	1020	1020	1014	1015	1019	14	12	3.18	3.22	3.62	3.91	2.41	1.96	42	40	40	38	38	146	143	141	143	147
S.D.	285	36	33	28	28	34	1017	1017	1017	1017	1017	1017	3	10	3.9	3.48	3.30	2.92	2.98	3.82	39	39	37	38	35	147	146	143	144	144
M.Hm.	240	51	26	24	23	40	1018	1018	1022	1022	1022	1016	7	10																
H.L.	1200	305	125	105	125	80	1008	1004	1008	1010	1008	1012	5	29																

* N.U.=night urine, voided between 10:30 P.M. and 7:30 A.M.

† F.U.=fasting urine, voided between 7:30 and 8:30 A.M.

Normal subjects

Four water tests were carried out in 3 normal subjects (Table 2). In each test the same general phenomena were noted. Little or no change occurred in the hematocrit—in two tests it rose by 1.5 and 2 per cent respectively (Fig. 2). By the end of the first hour following water ingestion the total serum base had fallen by 3 to 9 mEq./L (Figs. 3 and 4; Table 2).

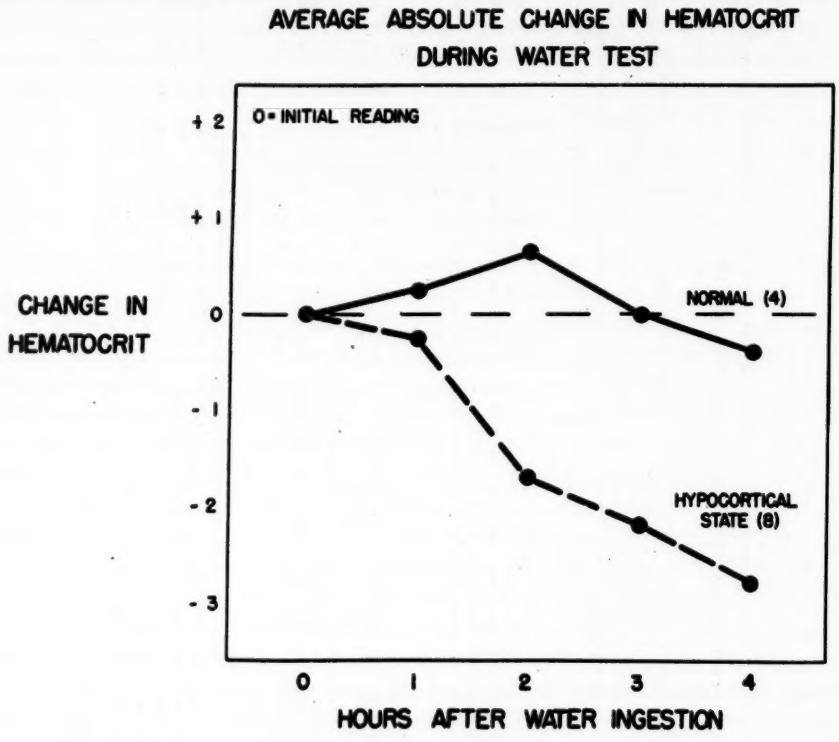


FIGURE 2

During the second hour a maximum diuresis of urine of low specific gravity occurred and at the end of the second hour the serum base had begun to rise. In the third and fourth hours diuresis gradually became less as the total base rose to normal levels. Antidiuretic assays of the pooled urine collected during the four hours of the test showed a consistent diminution in activity as compared with the "night urine," in those patients on whom tests were performed (Table 3).

These results are in good accord with Verney's studies (27) regarding the mechanism of water diuresis. He has shown that reduction in the level

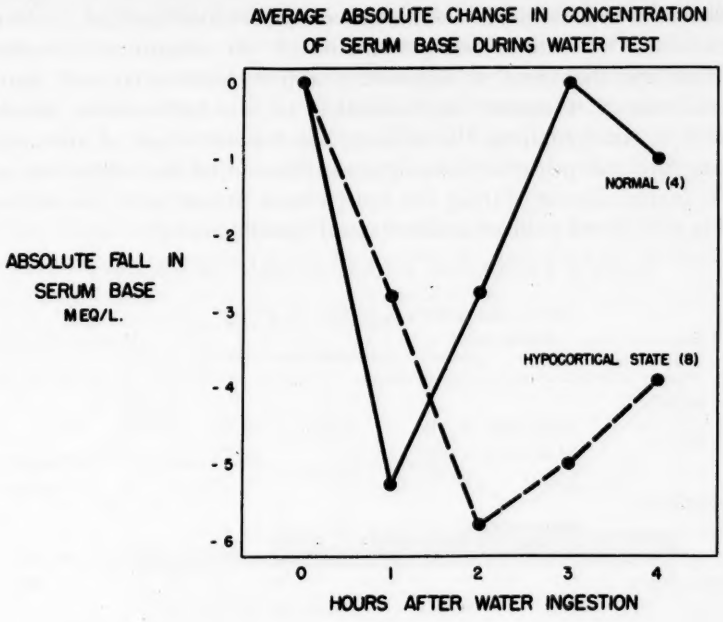


FIGURE 3

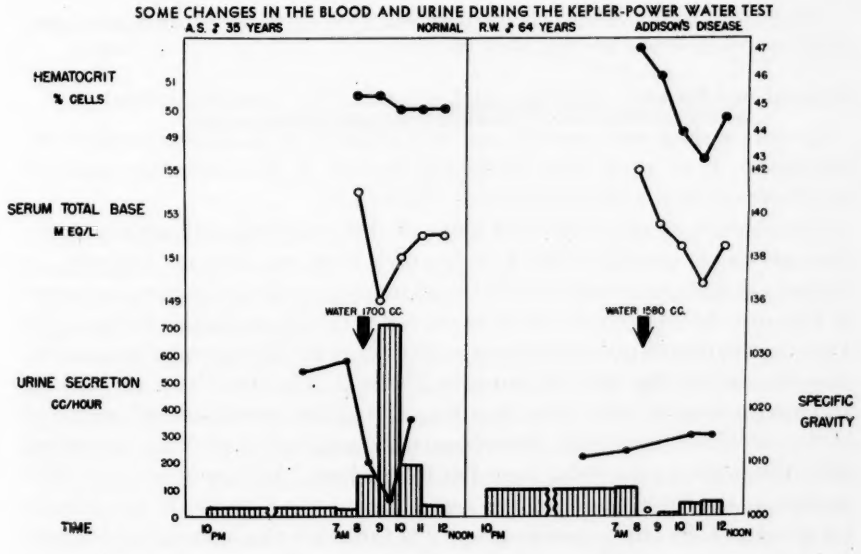


FIGURE 4

of osmotically active ions in the plasma causes an inhibition of the neurohypophysis. With diminished production of the antidiuretic hormone, the circulating hormone is apparently rapidly destroyed and diuresis takes place as a consequence of diminished tubular reabsorption of water. As water is removed from the plasma, the concentration of electrolytes increases and the posterior lobe again is stimulated to secrete the antidiuretic factor. Hence, during the fourth hour in our tests, the urine secreted is of reduced volume and increased specific gravity.

TABLE 3. ANTIDIURETIC ACTIVITY OF URINE CONCENTRATES

Case	Night urine	From 1 to 4 hours after ingestion of water	During diuresis
Normal subjects			
A.S.	Moderate (7.1)*	Slight (2.0)	—
F.G.	Moderate (4.56)	Nil (0:77)	—
L.B.	Slight (2.70)	Nil (1.20)	—
Addison's disease			
M.Ht.	Moderate (5.38)	Moderate (4.29)	—
C.S.	—	Moderate (5.43)	Nil (0.42)
Hypopituitarism			
G.T.(1)	Nil (0.45)	Moderate (5.51)	—
G.T.(2)	Slight (3.96)	Slight (3.65)	Nil (1.51)
S.D.	—	Moderate (4.43)	Nil (0.20)

* Each figure in parentheses indicates the difference in square inches between the areas of the control curve and the test curve (*see* text).

Adrenal insufficiency (primary, and secondary to hypopituitarism)

Complete tests were carried out in 9 patients with adrenal cortical insufficiency; 6 of these had Addison's disease, 3 had secondary adrenal insufficiency from a pituitary lesion (Table 1).

The changes in the blood and urine of these patients following the test dose of water showed striking differences from the normal subjects. A typical example, contrasted with the changes in a normal person, is shown in Figure 4. In all cases the hematocrit fell. The fall amounted to from 3 to 4 per cent in 6 patients; in 3, it was only 1 per cent. The average hematocrit response during the tests is shown in Figure 2. The total base also fell, in an amount ranging from 4 to 10 mEq./L, and the maximum fall occurred at the second or third hour. Reference to Figure 3 will show that, compared with the normal, the total base fell more slowly and recovery was only partial in the later hours of the test. In 8 of the 9 patients no diuresis occurred and the fall in specific gravity of the urine was insignificant (Table 2). In 1 patient (M.Ht.) a slight and incomplete diuresis did take place

and the specific gravity fell considerably. In 3 cases, the antidiuretic activity of the night urine was contrasted with that of the urine passed during the four hours of the water test (Table 3). In none did the latter have less antidiuretic activity than the night urine—in marked contrast to the urine of the normal subjects. Indeed, the night urine of G.T. had no measurable antidiuretic activity, whereas the urine following water ingestion had "moderate" activity.

From these results one may draw certain conclusions: 1) that water is absorbed adequately when given by mouth; 2) that the rate of absorption

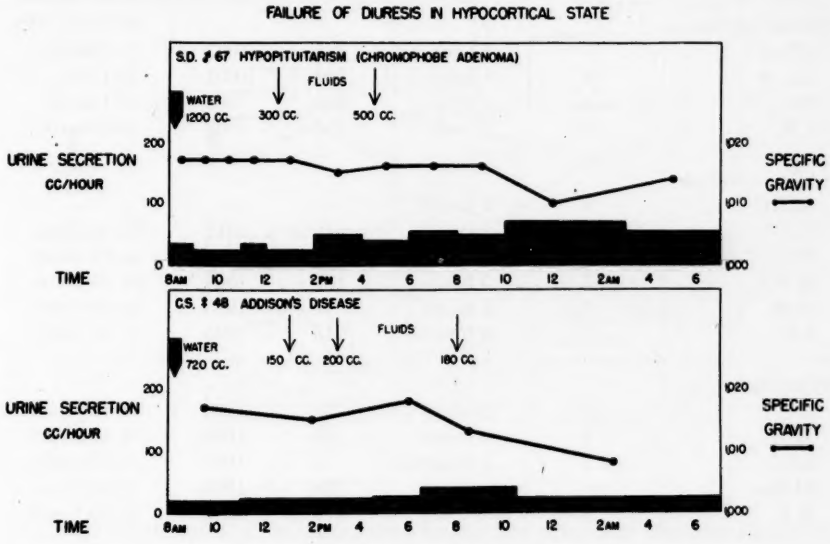


FIGURE 5

is slower than normal; 3) that the dilution of the plasma as measured by total base is of the same order as normal; 4) as measured by urinary assays, there is no reduction of antidiuretic activity in the urine passed in the four hours of the water test.

In brief, the physiologic conditions for inhibition of the posterior pituitary gland and for diuresis are present but neither of these occurs. It may be concluded that in adrenal insufficiency a disorder in the neurohypophyseal control of water excretion must be considered.

Late diuresis. Since no diuresis occurred at the time when expected (1–4 hours after the water dose) observations were made in 10 cases to determine if diuresis did occur later. In 3 patients no diuresis occurred during the twenty-four hours of observation, the rate of urine flow not exceeding 70

TABLE 4. FALL IN SERUM ELECTROLYTES AND TIME OF DIURESIS AFTER WATER INGESTION IN NORMAL PERSONS AND IN PATIENTS WITH ADDISON'S DISEASE AND HYPOPITUITARISM

	Max. fall in serum base after water ingestion		Diuresis		
			Urine		Time of diuresis after water ingestion
	Degree of fall, (mEq./L)	Time of fall	Max. flow, ml/hour	Sp. gr.	
Normal subjects					
A.S.-1	5	1 hour	735	1002	1–3 hours
A.S.-2	4	1 hour	710	1003	2–3 hours
F.G.	4	2 hours	630	1003	2–4 hours
L.B.	9	1 hour	730	1003	1–3 hours
Addison's disease					
M.Ht.	5	2 hours	—	—	—
C.S.	6	2 hours	40	1013	No diuresis
W.D.	9	3 hours	180	1006	6–13 hours
R.W.	5	3 hours	186	1009	14–24 hours
H.W.	9	3 hours	45	1015	No diuresis*
J.C.	6	2 hours	213	1005	6–10 hours
Hypopituitarism					
S.T.	6	2 hours	291	1005	6–12 hours
M.R.	5	2 hours	206	1006	6–15 hours
S.D.	5	3 hours	70	1010	No diuresis
M.Hm.	—	—	208	1005	8–14 hours
H.L.	—	—	182	1012	12–16 hours

* Fever and sore throat developed on day of the test; diaphoresis may account in part for absence of diuresis.

ml. per hour (Fig. 5 and Table 4). In the other 7, however, an increased rate of urine secretion was observed, varying from 180 to 290 ml. per hour. Three examples of this late diuresis are shown in Figure 6. It will be noted that when the rate increased, the specific gravity of the urine tended to fall. The time at which the late diuresis occurred varied from patient to patient. In 1 patient (M.R.) it began six hours after the water dose; in another, twelve hours afterward. In 2 cases in which repeat observations were made throughout the twenty-four hours, diuresis began at the same time as in the initial tests and lasted as long. It seems likely that when diuresis takes place the time of its occurrence is a characteristic of the individual case of cortical insufficiency.

In 3 patients antidiuretic assays were made of the urine passed during the time of late diuresis (Table 3) and in each case no activity could be shown. The urine voided during the water test on the other hand showed moderate antidiuretic activity in 2 patients and slight in 1. It would thus seem that when late diuresis occurred, it was accompanied by a lessened excretion of antidiuretic substance in the urine.

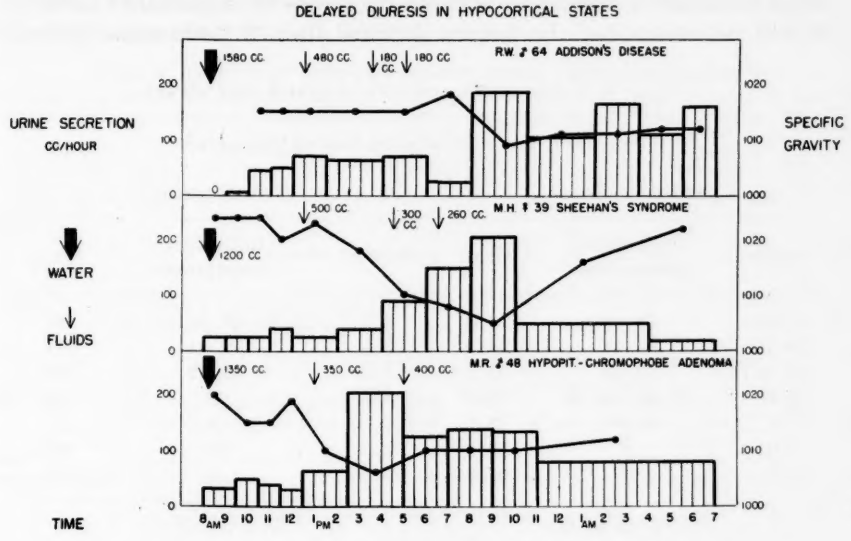


FIGURE 6

Antidiuretic activity of the serum. Using the modified method of Birnie *et al.* previously described, the antidiuretic activity of serum after a period of fourteen hours of water restriction was measured in 7 normal subjects and in 7 patients with Addison's disease. In each group there were 3 males and 4 females. The normal subjects were laboratory staff members and medical students. Six of the patients with Addison's disease had been admitted to the hospital for routine checking and re-implantation of DCA pellets; one (E.C.) had been admitted in crisis due to an infection, but had been convalescent for two weeks. Since it is known that there is a tendency to water retention and possibly increased neurohypophyseal activity towards the end of the menstrual cycle, the tests were carried out in the women between the ninth and eighteenth day.

Results: In the rat assay for serum antidiuretic activity (*see* methods) the percentage excretion of the water load at ninety minutes was taken as the value for comparison in each group. The individual observations made

on the sera of each group are presented in Table 5. In 14 control observations in which rats were given normal saline intraperitoneally as the test substance, the mean percentage excretion of the load at ninety minutes was 68.79 (S.E. ±2.35). Serum from the normal subjects gave a mean value of 55.00 (S.E. ±1.60); for serum from patients with Addison's disease the mean value was 37.03 (S.E. ±5.96). In the absence of correction for unequal variance the differences between the means are significant (Table 5). It will be noted that the figures obtained from 2 Addisonian patients

TABLE 5. ANTIDIURETIC ACTIVITY OF SERUM (RAT ASSAY)

Percentage excretion of water load at 90 minutes					
Addison's disease			*Normal patients*		
Subject	Individual observations	Mean	Subject	Individual observations	Mean
W.D.	18, 28	23.0	A.S.	56, 48, 64, 53, 65, 53	58
M.Ht.	64, 47	55.5	F.G.	59, 51	55
D.M.D.	28, 39	33.5	L.B.	54, 62	58
R.W.	28, 28, 23, 20	24.7	A.A.	48	48
C.S.	20, 25	22.5	P.H.	51	51
E.R.	39	39.0	P.B.	60	60
E.C.	61	61.0	R.B.	55	55
37.03 ± 5.96 [7]			55.0 ± 1.60 [7]		
Saline controls 68.79 ± 2.35 [14]					

A significant difference is encountered between the means for Addisonian patients and normal patients ($P = < .01$) and between normal patients and saline controls ($P = < .02$) when the 't' test is applied, in the absence of correction for unequal variance.

(M.Ht. and E.C) are much higher than the others and fall within the range of the figures for the normal subjects. The water test of M.Ht. showed the characteristic Addisonian response but the degree of deficiency in the excretion of the water dose was much less than in any other patient studied (Table 2). M.Ht. excreted 45 per cent of the water dose in the four hours of the test; the range for the other patients was from 8 to 29 per cent. In patient E.C. a water test was not carried out. If these 2 high results are excluded from the Addisonian group, correction for unequal variance by the method of Cochran and Cox (38) shows significant differences between the means.

These results indicate a higher than normal titer of antidiuretic material in the serum of Addisonian patients, and confirm the observations of Birnie

et al.(24) in adrenalectomized rats. Lloyd and Lobotsky (39) have reported similar findings in 2 patients with Addison's disease.

The effects of treatment on the response to water. The effects of various forms of treatment on the water test have been observed in 6 patients, 3 with Addison's disease and 3 with hypopituitarism. The results and details of therapy are presented in Table 6. Treatment with Lipo-adrenal Cortex (2 cases) and DCA (1 case) failed to alter the response to water. A depot of testosterone macrocrystals, 200 mg. given to G.T. intramuscularly five

TABLE 6. EFFECTS OF VARIOUS FORMS OF THERAPY ON THE WATER TEST

Patient	Treatment	Dose of water (ml.)	Urinary volumes in ml.					Per cent of water dose excreted	Value "A"
			Night urine	1	2	3	4		
				(hrs. after water)					
M.Hm. Hypopit.	nil	1200	240	26	24	23	40	9.4	—
	10 mg. DCA 10 hrs. before water	1200	390	34	31	21	26	9.3	—
G.T. Hypopit;	nil	1280	440	32	28	35	30	9.7	3
	200 mg. testoterone macrocrystals injected 5 days prior to test	1320	250	52	60	110	100	24.4	19
M.R. Hypopit.	100 ml. 5% glucose (control) i.v.	1350	370	48	48	39	28	12.0	14
	100 ml. cortical extract i.v.	1350	537	61	126	59	63	21.4	9
M.H. Addison's disease	3 Gm. NaCl daily	1000	228	52	160	148	92	45.2	13
	160 workunits Lipo-adrenal Cortex i.m. daily for 3 days.	1000	122(?)	35	122	202	38	39.7	171(?)
C.S. Addison's disease	nil	720	290	—	—	56	—	7.8	4
	240 work units Lipo-adrenal Cortex i.m. daily for 4 days.	720	260	18	12	10	7	6.5	7
J.C. Addison's disease	nil	1100	155	34	30	27	24	11.4	6
	200 mg. compound E	1100	592	114	340	285	50	71.7	42
	Two weeks after compound E and 10 days after 250 mg. DCA implant	1100	638	58	100	124	72	32.0	2

days before the water test was repeated, may have been responsible for the observed increase in hourly urinary volumes, but the increase was relatively little and fell far short of a normal response. One hundred ml. of aqueous cortical extract (Eschatin) given intravenously as part of the water dose and simultaneously with the ingestion of water also failed to produce a normal diuresis, although the maximum hourly volume rose from 48 to 126 ml. On the other hand, cortisone acetate given to J.C. caused a normal type of diuresis. Cortisone was given in two 100-mg. doses; one, ten and a half hours, the other, two and a half hours before the dose of water. The value "A" increased from 6.5 to 41.7. A repeat control test carried out

two weeks after cortisone administration was similar in type to the first control.

The failure to correct the water retention with cortical extract and small doses of DCA is in keeping with the findings of other workers (4, 6, 40). A large dose of DCA, 120 mg. in beeswax and oil given intramuscularly, was reported by Levy (6) to have produced temporarily a normal diuretic response to water in 1 case of Addison's disease.

DISCUSSION

These studies indicate that in human adrenal insufficiency when a large dose of water is given it enters the blood to produce a hydremia. How much of the water enters the various fluid compartments cannot be determined from our data. The dilution of the serum total base so produced is of the same order as in the normal subject; but whereas in the latter it induces a diuresis, in cortical insufficiency it fails so to do. We must then conclude that the fault in water excretion is at the renal level. All previous studies on renal function in untreated clinical adrenal insufficiency (16–19) have consistently demonstrated diminished glomerular filtration. A slow rate of filtration might lead to increased tubular reabsorption since the volume of filtrate reaching tubular cells per unit of time would be reduced and may lead to more effective reabsorption. If a slow rate of glomerular filtration were solely responsible for the lack of prompt water diuresis when hydremia exists, a later diuresis would be unexpected; but such occurs in the majority of patients and the increased rate of urine flow is accompanied by a fall in specific gravity. Further, treatment of adrenal insufficiency in man by deoxycortone (19) and in rats by salt (20, 21) causes essentially normal filtration rates, but the prompt diuretic effect of water is still absent. We are thus led to suspect that augmented tubular function rather than diminished glomerular filtration alone is playing an important part in determining the water retention of Addison's disease. This is in bearing with the conclusions of Boss *et al.* in relation to the same problem in adrenalectomized rats (15.)

Turning now to the finding of increased antidiuretic activity in the fasting serum of patients with adrenal insufficiency and of adrenalectomized animals, it is important to discuss the source of this factor. Our studies contribute nothing on this point. Birnie *et al.* (24, 25, 41), however, have shown that the increased titer of antidiuretic substance in the serum of adrenalectomized rats disappears after complete hypophysectomy. They have demonstrated in the test animals a chloruretic effect of the serum of adrenalectomized rats and have also shown that the serum antidiuretic substance is quickly inactivated by neutral thioglycollic acid, as is pitressin.

From the above evidence it would seem likely that the antidiuretic factor is of posterior pituitary origin. There is, however, some difficulty in accepting this conclusion unreservedly. Verney (27) has shown that the level of osmotically active ions in the plasma plays a prime role in the control of neurohypophyseal activity. When the level is raised, increased secretion of posterior pituitary hormone takes place; when the level is reduced, inhibition occurs. In adrenal insufficiency the total serum base in the fasting state is reduced compared with normal. In our studies the mean fasting total base value in the normal subjects was 152 mEq./L; in patients with Addison's disease 144.5; and in the hypopituitary group 145.3. With such subnormal levels of serum base one may reasonably expect a *diminished* activity of the neurohypophysis. Nevertheless an increased titer of antidiuretic substance is found in the fasting serum in Addison's disease. One other difficulty concerns the amount of antidiuretic material in the fasting serum. In terms of pitressin, it is of the order of 0.25–0.5 m.u. per ml. serum; and Lloyd reports quantities up to 5 m.u. per ml. (39). With such quantites of posterior pituitary hormone in the blood the patients should readily show the other pharmacologic actions of pitressin besides antidiuresis, namely, purging, abdominal colic and pallor. These symptoms may indeed be present but, save in crisis, are not striking features of Addison's disease. With the exception of pallor, none of these symptoms was noted at the time of the antidiuretic assays on the fasting sera.

Until the antidiuretic material in the serum of patients with adrenal deficiency has been shown unequivocally to have all the characteristics of the posterior pituitary hormone, the exact relationship between the adrenal cortex and the neurohypophysis cannot be defined. Studies to this end are in progress.

Assuming for the time being that the antidiuretic factor is of posterior pituitary origin we can proceed to discuss the problem of delayed diuresis with reference to the observations reported in this paper.

When a fasting Addisonian is given a load of water, there is already present in the blood an increased titer of antidiuretic substance and it is difficult to avoid the conclusion that this plays an important part in delaying diuresis. In the normal fasting subject the ingestion of water is followed within minutes by an increased rate of urine flow. To allow this to occur, not only has the output of hormone from the posterior lobe to diminish or cease, but the hormone circulating in the blood has to be rendered ineffective or removed. Whether one or both of these mechanisms is at fault in adrenal insufficiency cannot be stated with certainty but the evidence available will now be examined.

Studies reported in this paper show that the condition necessary for

inhibition of posterior pituitary secretion, diminished osmotic pressure of the plasma electrolytes, exists in the patient with adrenal insufficiency who has been given a large volume of water; but the urinary output of antidiuretic material in the four hours following water ingestion is not diminished (Table 3). This situation of increased or maintained neurohypophyseal activity would occur if the posterior pituitary gland were affected by some influence other than the level of the plasma electrolytes; and such a possibility exists. Baez *et al.* (42) in their investigation of ferritin (VDM) found it possessed antidiuretic properties when administered to normal dogs and rabbits undergoing a water diuresis. Ferritin, a substance of hepatic origin found in the later stages of shock, is thus an agent which could stimulate the posterior pituitary gland. That such an agent may be present in the blood of a patient with Addison's disease has also been shown by Shorr. In one patient who was in crisis, increased quantities of ferritin were found (43). But whether an increased level of ferritin is characteristic of patients with Addison's disease not in crisis is not known. It seems more likely to be associated with the shock-like state of the Addisonian crisis.

Evidence that the posterior pituitary gland is not overactive in adrenal insufficiency comes from the studies of Gersh and Grollman (12). In adrenalectomized rats these workers have found indications of increased tubular reabsorption but could find in the neurohypophysis none of the histologic evidences of increased activity which Gersh (44) had previously shown to be characteristic of dehydration.

Two observations made in this study seem to have a bearing on this problem. (A) Although water diuresis in the normal subject was associated with a diminished output of antidiuretic substance, in patients with cortical insufficiency there was neither a diuresis nor diminished antidiuretic activity in the urine in the four hours following water ingestion. (B). In cortical insufficiency a delayed diuresis occurred not infrequently and the urine passed at the time of diuresis had no antidiuretic activity. It would seem reasonable to conclude that the presence or absence of diuresis is related to the presence of an antidiuretic factor in the urine, presumably of posterior pituitary origin. If this hormone were not promptly rendered ineffective or removed, a prompt diuresis would not follow ingestion of water even though secretion of the hormone from the neurohypophysis has ceased. It is possible that the mechanism for removing posterior pituitary hormone from the blood is faulty in adrenal insufficiency. Normally it is promptly rendered ineffective when conditions for diuresis are present; in Addison's disease its destruction in vivo might be slow and a number of hours elapse before a sufficient amount is destroyed to allow diuresis to take place.

This hypothesis that the cortical hormones play an essential role in the

prompt removal or inactivation of posterior pituitary hormone has recently received some support from Gaunt's laboratory. The liver of the rat on the fifth day after adrenalectomy shows a diminished ability to inactivate pitressin (45). Such a state would inevitably cause an accumulation of posterior pituitary hormone and lead to antidiuresis. There is, however, another factor of some importance. Gaunt and his group have shown that adrenalectomized rats have an increased sensitivity to pitressin (5), and Mary Lockett working in Glasgow has extended this in adrenalectomized dogs (46). The combination of diminished destruction and in-

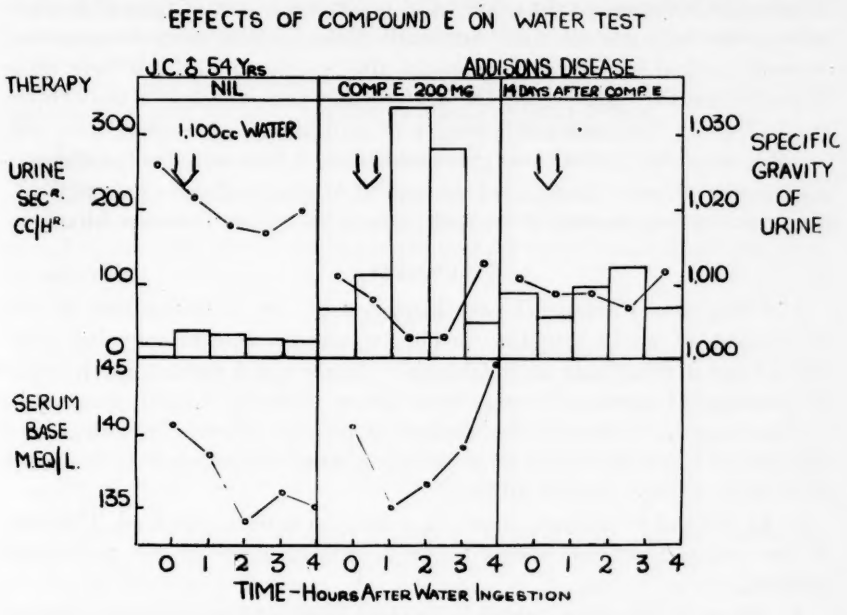


FIGURE 7

creased sensitivity to posterior pituitary hormone would give rise to functional hyperpituitarism of the posterior-lobe type—the chief characteristic of which would be antidiuresis.

Apart from the present investigation, studies in man on the relationship of antidiuretic activity of blood to adrenal function have been carried out only by Lloyd and Lobotsky (39). These authors were able in a variety of clinical states to show that a high titer of antidiuretic substance in the serum was related to a low output of urinary corticoids, as in Addison's disease; and that low titers of serum antidiuretic substance were associated with normal or high corticoid excretion and high urinary volumes, as in

diabetes insipidus. As in the present investigation, the source of antidiuretic material found by Lloyd is not known.

Regarding the correction of the water fault in clinical adrenal insufficiency, replacement therapy until recently has not been successful. An increase in filtration rate towards normal can be produced by DCA or cortical extract (16, 17, 19), but the characteristic water retention persists. This has been confirmed in the present study. One hundred ml. of extract given intravenously and simultaneously with water ingestion did not cause a normal diuresis, although hourly urinary volumes were slightly increased (Table 6). Cortisone on the other hand produced a normal type of diuresis when given to a patient with Addison's disease whose control water test showed marked retention. Two weeks after cortisone and ten days after implantation with 250 mg. of DCA, the water test reverted to the control type (Fig. 7). Unfortunately, assays of antidiuretic substance were not possible when this patient was given cortisone. It thus remains to be shown if cortisone corrects the delayed diuresis in Addison's disease by inactivating or hastening removal of the antidiuretic substance from the blood.

SUMMARY

1. Using the Robinson-Power-Kepler test, an investigation of the mechanism of water retention in clinical adrenal insufficiency has been carried out in 6 patients with Addison's disease and 5 patients with hypopituitarism. Absorption of water from the gut, although slightly delayed, is not an important cause of the absence of prompt diuresis. The degree of dilution of the serum total base following water ingestion is of the same order as in normal control subjects.

2. In 7 out of 10 patients observed, a delayed diuresis occurred. The time of the delayed diuresis seems to be a characteristic of the individual patient.

3. Assays of the urine passed in the four hours of the water test showed evidence of moderate antidiuretic activity, whereas urine passed during the delayed diuresis showed little or no antidiuretic activity. In the fasting serum of Addisonian patients there was a marked increase of antidiuretic substance.

On the basis of these findings it is concluded that an antidiuretic substance in the blood to an important extent is responsible for the absence of prompt diuresis in adrenal insufficiency. Since the phase of late diuresis is associated with little or no urinary antidiuretic activity, it is suggested that the rate of removal of the antidiuretic substance in Addison's disease is delayed.

4. Cortisone restored the time and degree of diuresis to normal in the only patient with Addison's disease to whom it was given.

On the assumption that the antidiuretic material found in the urine and blood are of neurohypophyseal origin, a hypothesis is suggested that the adrenal hormones of the cortisone type may be concerned with the inactivation of circulating posterior pituitary hormones.

Acknowledgments

The major part of this work was carried out in Dr. George W. Thorn's service at the Peter Bent Brigham Hospital, Boston, during the tenure of a Commonwealth Fund Fellowship in 1948–49. The author gratefully acknowledges the facilities granted him there. He is indebted to Dr. George Thorn for his encouragement and for his criticism of the manuscript, to Mrs. Connie Forsham for biochemical assistance, to Mrs. Nancy Meegan for assistance with the antidiuretic assays, and to Dr. Mary F. Lockett for the statistical analysis.

The early observations were made in Glasgow when the work was supported by an expenses grant from the Medical Research Council of Great Britain.

Addendum

Since this paper was submitted for publication it has been found that the normal diuretic response to water which cortisone produces in patients with adrenal insufficiency is associated with a substantial reduction in the antidiuretic activity of the fasting serum.

REFERENCES

1. Marshall, E. K., Jr., and Davis, David M.: The influence of the adrenals on the kidneys, *J. Pharmacol. & Exper. Therap.* **18:** 525, 1916.
2. Rowntree, L. G., and Snell, A. M.: A Clinical Study of Addison's Disease, Mayo Clinic Monographs, Philadelphia, W. B. Saunders Company, 1931.
3. Robinson, F. J.; Power, M. H., and Kepler, E. J.: Two new procedures to assist in the recognition and exclusion of Addison's disease, *Proc. Staff Meet., Mayo Clin.* **16:** 577, 1941.
4. Gaunt, R.: Animal experiments relating to water diuresis tests for adrenal insufficiency, *J. Clin. Endocrinol.* **6:** 595, 1946.
5. Gaunt, R.; Birnie, James H., and Eversole, W. J.: Adrenal cortex and water metabolism, *Physiol. Rev.* **29:** 281, 1949.
6. Levy, M. S.; Power, M. H., and Kepler, E. J.: The specificity of the "water test" as a diagnostic procedure in Addison's disease, *J. Clin. Endocrinol.* **6:** 607, 1946.
7. Reforzo-Membrives, J.; Power, M. H., and Kepler, E. J.: Studies on the renal excretion of electrolytes in cases of Addison's disease, *J. Clin. Endocrinol.* **5:** 76, 1945.
8. Silvette, H., and Britton, S. W.: Effects of adrenalectomy and cortico-adrenal extract on renal excretion and tissue fluids, *Am. J. Physiol.* **104:** 399, 1933.
9. Gaunt, R.: Water diuresis and water intoxication in relation to the adrenal cortex, *Endocrinology* **34:** 400, 1944.
10. Shipley, R. A.: The cause of abnormal retention of ingested water in adrenalectomized rats, *Endocrinology* **36:** 118, 1945.
11. Harrison, H. E., and Darrow, D. C.: Renal function in experimental adrenal insufficiency, *Am. J. Physiol.* **125:** 631, 1939.
12. Gersh, I., and Grollman, A.: Kidney function in adrenal cortical insufficiency, *Am. J. Physiol.* **125:** 166, 1939.

13. WHITE, H. L.; HEINBECKER, P., and ROLF, D.: Renal function in hypophysectomized dogs, *Proc. Soc. Exper. Biol. & Med.* **46:** 44, 1941.
14. WHITE, H. L.; HEINBECKER, P., and ROLF, D.: Some endocrine influences on renal function and cardiac output, *Am. J. Physiol.* **149:** 404, 1947.
15. BOSS, W. R.; BIRNIE, J. H., and GAUNT, R.: Renal function in untreated adrenalectomized rats, *Fed. Proc.* **8:** 13, 1949.
16. TALBOTT, J. H.; PECORA, L. J.; MELVILLE, R. S., and CONSOLAZIO, W. V.: Renal function in patients with Addison's disease and in patients with adrenal insufficiency secondary to pituitary panhypofunction, *J. Clin. Investigation* **21:** 107, 1942.
17. MCGAVACK, T. H.; SACCONE, A.; VOGEL, M., and HARRIS, R.: Craniopharyngioma with panhypopituitarism: case report with clinical and pathological study, *J. Clin. Endocrinol.* **6:** 776, 1946.
18. WATERHOUSE, C., and KEUTMAN, H.: Renal function in adrenal insufficiency, *J. Clin. Investigation* **27:** 372, 1943.
19. SANDERSON, P. H.: Renal function in Addison's disease, *Clin. Sc.* **6:** 197, 1946–48.
20. LOTSPEICH, W. D.: Glomerular filtration rate in the adrenalectomized, salt fed rat, *Fed. Proc.* **7:** 74, 1949.
21. FRIEDMAN, S. M.; MACKENZIE, K. R., and FRIEDMAN, C. L.: Renal function in the adrenalectomized rat, *Endocrinology* **43:** 123, 1948.
22. STARLING, E. H., and VERNEY, E. B.: The secretion of urine as studied on the isolated kidney, *Proc. Roy. Soc., London* s.B., **97:** 321, 1924–25.
23. MARTIN, S. J.; HERRLICH, H. C., and FAZEKAS, J. F.: Relation between electrolyte imbalance and excretion of an antidiuretic substance in adrenalectomized cats, *Am. J. Physiol.* **127:** 51, 1939.
24. BIRNIE, J. H.; JENKINS, R.; EVERSOLE, W. J., and GAUNT, R.: An antidiuretic substance in the blood of normal and adrenalectomized rats, *Proc. Soc. Exper. Biol. & Med.* **70:** 83, 1949.
25. BIRNIE, J. H.; EVERSOLE, W. J.; BOSS, W. R.; OSBORN, C. M., and GAUNT, R.: Properties of the anti-diuretic substance in the blood of normal and adrenalectomized rats, *Fed. Proc.* **8:** 12, 1949.
26. GRAHAM, J. D. P., and SLESSOR, A.: Unpublished.
27. VERNEY, E. B.: Agents determining and influencing the function of the pars nervosa of the pituitary, *Brit. M. J.* **2:** 119, 1948.
28. SCHALES, O., and SCHALES, S. S.: A simple and accurate method for the determination of chlorides in biological fluids, *J. Biol. Chem.* **140:** 879, 1941.
29. ASPER, S. P.; SCHALES, O., and SCHALES, S. S.: Importance of controlling pH in the Schales and Schales method of chloride determination, *J. Biol. Chem.* **148:** 779, 1947.
30. VAN SLYKE, D. D.: The manometric determination of urea in blood and urine by the hypobromite method, *J. Biol. Chem.* **83:** 449, 1929.
31. ARCHIBALD, R. M.: Colorimetric determination of urea, *J. Biol. Chem.* **157:** 507, 1945.
32. PETERS, J. P., and VAN SLYKE, D. D.: Quantitative Clinical Chemistry. Baltimore, The Williams & Wilkins Co., 1943, vol. 2, p. 941.
33. SUNDERMAN, F. W.: Measurement of total serum base, *Am. J. Clin. Path.* **15:** 219, 1945.
34. GORBMAN, A.: Ultrafiltration of urine for collection and biological assay of excreted hypophyseal hormones, *Endocrinology* **37:** 177, 1945.
35. HAM, G. C., and LANDIS, E. M.: A comparison of pituitrin with the anti-diuretic substance found in human urine and placenta, *J. Clin. Investigation* **21:** 455, 1942.

36. BURN, J. H.: Estimation of the anti-diuretic potency of pituitary (posterior lobe) extract, *Quart. J. Pharm. & Pharmacol.* **4**: 517, 1931.
37. GILMAN, A., and GOODMAN, L.: The secretory response of the posterior pituitary to the need for water conservation, *J. Physiol.* **90**: 113, 1937.
38. SNEDECOR, GEORGE W.: Statistical Methods. Ames, Iowa, The Iowa State College Press, 1946.
39. LLOYD, C. W., and LOBOTSKY, J.: Serum antidiuretic substances and urinary corticosteroid in the human, *J. Clin. Endocrinol.* **10**: 318, 1950.
40. WUNDERMAN, D. C., and LEVY, M. D.: Application of Robinson-Power-Kepler tests for diagnosis in two cases of Addison's disease, *Med. Record*, Houston **42**: 578, 1948.
41. JENKINS, R., and BIRNIE, J. H.: An anti-diuretic substance in the blood of dehydrated and hydrated rats. *Anat. Rec.* **103**: 543, 1949.
42. BAEZ, S.; MAZUR, A., and SHORR, E.: Hepatorenal factors in circulatory homeostasis. XX. Antidiuretic action of hepatic vasodepressor, VDM (ferritin), *Am. J. Physiol.* **162**: 198, 1950.
43. SHORR, E.: Personal communication.
44. GERSH, I.: The structure and function of the parenchymatous cells in the neurohypophysis of the rat, *Am. J. Anat.* **64**: 407, 1939.
45. GAUNT, R.: quoted by Roemmelt, J. C. and others. *Am. J. Physiol.* **159**: 124, 1949.
46. LOCKETT, MARY F.: Variations in the sensitivity of adrenalectomized dogs to the anti-diuretic hormone. Ciba Foundation Conference, London, Jan. 1951. (In preparation.)

TESTOSTERONE CYCLOPENTYLPROPIONATE: A LONG-ACTING ANDROGEN

CHARLES W. LLOYD, M.D. AND JOAN FREDERICKS, B.A.

From the Department of Obstetrics, Syracuse University College of Medicine, Syracuse, N. Y.

A STUDY of the duration of action of testosterone crystals and of testosterone adsorbed on aluminum phosphate has recently been published by Eversole, Leathem, and Schraer (1). In a footnote to this paper, the use in this laboratory of these materials in humans has been described. Both the macrocrystalline suspension of testosterone and the testosterone adsorbed on aluminum phosphate had periods of activity in the human which were considerably longer than the duration of effectiveness of testosterone propionate, but difficulties of administration rendered each of these preparations impractical for clinical use. The cyclopentylpropionic acid ester of testosterone has been found by Ott and his co-workers (2) to have a duration of effectiveness, as measured by the response of the accessory sexual tissues of the castrated male rat, which is markedly longer than that of testosterone propionate. The present report is concerned with a comparison, in hypogonadal men, of the effectiveness of oil solutions of this material and of testosterone propionate.

METHODS AND MATERIALS[1]

A single intramuscular injection of a preparation of testosterone cyclopentylpropionate in oil (hereafter called TCP), 30 mg. per cc., was used. For comparison, a single injection of testosterone propionate in oil (hereafter called TP), 25 mg. per cc., was given in a comparable dose. The subjects used for testing the material consisted of 7 male hypogonadal patients, and 1 young male castrate. The effectiveness of the androgen was evaluated by daily recordings of spontaneous erections, nocturnal emissions, and in the case of the castrate, by frequency of sexual intercourse. Subjective evaluation of physical strength was also utilized as an indication of androgen effect. On a few occasions, 17-ketosteroid excretion following injection of the androgen was estimated by the method of Drekter *et al.* (3).

Received for publication December 21, 1950.

[1] Testosterone propionate and testosterone cyclopentylpropionate used in these studies were supplied through the courtesy of Dr. H. F. Hailman of the Upjohn Company, Kalamazoo, Michigan.

RESULTS

Injection of TP in doses varying from 100 to 300 mg. resulted in an androgen effect for as long as fourteen days. The duration and intensity of effect both varied directly with the dose. In no patient was adequate androgenic activity detectable after fourteen days.

Forty-five injections of TCP were given to the 8 patients. None of the patients had any untoward reactions. The adequate dose for maintenance of a sense of well-being, good physical strength, and of a moderate amount of spontaneous sexual activity (1–4 erections daily) varied from 50 to 300 mg. The eunuchoid patients required up to 200 mg. a month to produce normal physical strength and daily spontaneous erections. For maintenance of full sexual potency, the castrate required 300 mg. a month. In these patients the maximum androgenic effect was reached in from three to four days, was maintained at this level for approximately three weeks, then gradually decreased until at the end of four weeks a definite deficiency was apparent. The dose necessary to maintain adequate physical strength was considerably below that required for normal sexual potency.

The 17-ketosteroid excretion was studied following the administration of TP or TCP to 2 subjects. The results obtained in 1 of these hypogonadal patients are shown in Figure 1. The excretion pattern of the second patient studied following injection of TP or TCP was similar to that shown in

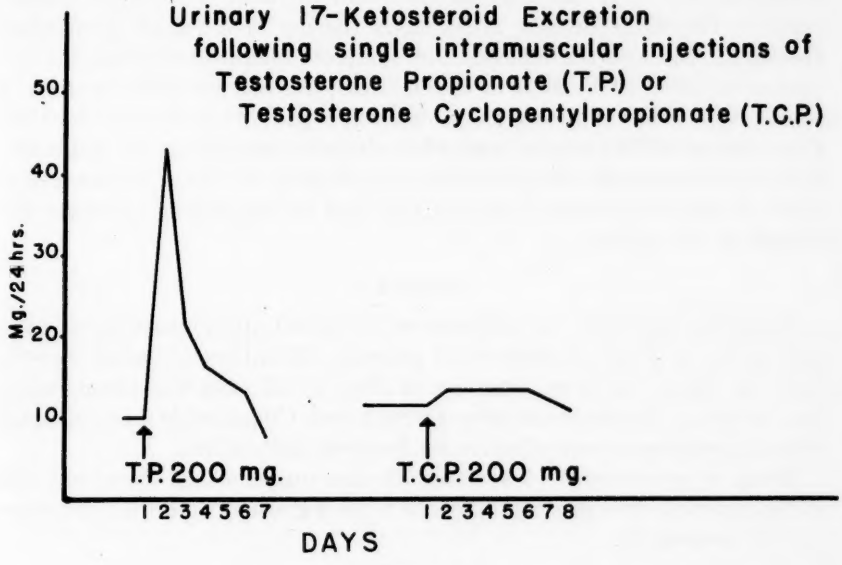


FIGURE 1

Figure 1. The 17-ketosteroid excretion following injection of testosterone propionate reached a high level during the twenty-four hours after injection and then fell to control levels in a few days. Following the administration of a comparable dose of testosterone cyclopentylpropionate no significant increase in 17-ketosteroid excretion occurred. In the other patient studied, a second injection of TCP given one week after the initial TCP injection caused a summation of excretion so that a higher level was reached than after the single injection alone; but the total 17-ketosteroid excretion following the two doses of TCP did not exceed the excretion following one dose of TP.

DISCUSSION

Clinical evaluation of this new androgen indicates a duration of effectiveness which is at least twice, and in some instances three to four times, longer that that of testosterone propionate. The 17-ketosteriod excretion studies suggest the possibility that the lesser effectiveness of TP may be the result of a more rapid rise in circulating steroid level than can be efficiently utilized by the organism, whereas TCP may be absorbed at a rate which is sufficiently slow to permit efficient utilization.

For androgen substitution therapy, this material has decided usefulness. Testosterone propionate has the disadvantage of requiring relatively frequent injections. Oral androgen therapy usually requires the ingestion of comparatively large amounts of the hormone with consequent greater expense. Buccal absorption necessitates the use of a vehicle permitting absorption through the mucosa. For complete buccal absorption the androgen tablet must be kept in contact with the mucous membrane for a considerable time, a procedure which some patients find objectionable. Testosterone pellets require somewhat elaborate equipment for implantation, and occasionally the pellet may extrude from the site of implantation. None of these objections holds for the type of long-acting androgen described in this paper.

SUMMARY

Forty-five injections of testosterone cyclopentylpropionate in oil have been given to 8 male hypogonadal patients. Effectiveness varied directly with the size of the dose. Duration of effect in all cases was twenty-eight days or more. No untoward effects were noted. Comparable doses of testosterone propionate were effective for fourteen days or less.

Study of excretion of 17-ketosteroids also suggests that the absorption of testosterone cyclopentylpropionate is more gradual than that of testosterone propionate.

REFERENCES

1. Eversole, W. J.; Leathem, J. H., and Schraer, H.: Testosterone preparations with prolonged activity, *Endocrinology* **47**: 448–453 (Dec.), 1950.
2. Ott, A. C.; Kuizenga, M. H.; Lyster, S. C., and Johnson, B. A.: Personal communication.
3. Drekter, I. J.; Pearson, S.; Bartczak, E., and McGavack, T. H.: A rapid method for the determination of total urinary 17-ketosteroids, *J. Clin. Endocrinol.* **7**: 795–800 (Dec.) 1947.

CHANGES IN RESPONSIVENESS TO INSULIN INDUCED BY DESOXYCORTICOSTERONE, IN DIABETES MELLITUS*†

HYMAN J. ZIMMERMAN, M.D.,‡ ALVIN E. PARRISH, M.D. AND LOUIS K. ALPERT, M.D.

From the Departments of Medicine, Veterans Administration Hospital and the George Washington University School of Medicine, Washington, D. C.

PREVIOUS study of a group of patients with diabetes mellitus, in whom liver biopsies were performed, revealed a high degree of correlation between fatty metamorphosis in the liver and insulin-insensitivity (1). Analysis of the available data suggested that the two phenomena were related to an independent common etiologic factor, rather than that the liver derangement was directly responsible for the insulin-insensitivity (1).

The development of fatty infiltration of the liver and insulin-insensitivity, which has been demonstrated following the administration of anterior pituitary extract to animals (2), raised the possibility that at least one of factors responsible for the insulin-insensitivity in these human diabetics might be of pituitary origin (1). The demonstration by Cheng and Sayers (3) that desoxycorticosterone acetate increased the sensitivity to insulin in rats, presumably through the inhibition of the output of pituitary adrenocorticotropic hormone, led to the investigation of the effect of this steroid on insulin-sensitivity in human diabetes (4). The present study includes an extension of the previous observations in insulin-insensitive diabetes as well as an investigation of the effect of desoxycorticosterone on the responsiveness to insulin in the insulin-sensitive diabetic patient.

METHODS AND PROCEDURES

Fifteen patients with clinically well controlled diabetes mellitus were studied. Insulin-sensitivity was evaluated before and after the administra-

Received for publication August 31, 1950.

* Presented at the Annual Meeting of the Association for the Study of Internal Secretions, San Francisco, California, June 24, 1950.

† Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

‡ Present address: Veterans Administration Hospital, Omaha, Nebraska.

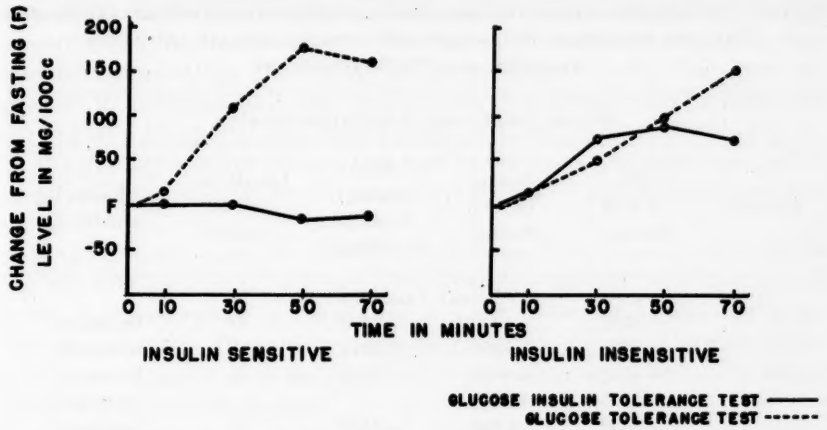


FIG. 1. Insulin sensitivity as measured by the Himsworth glucose-insulin tolerance test. The area between the glucose tolerance and the glucose-insulin tolerance curves is used as a measure of sensitivity to insulin.

tion of desoxycorticosterone acetate by the use of the Himsworth glucose-insulin tolerance test (5a). Glucose was administered orally and crystalline insulin intravenously, in doses of 30 Gm. and 5 units per square meter of body surface, respectively. The blood sugar was measured in the fasting state and at ten, thirty, fifty and seventy minutes after the glucose and insulin were given. Comparison of the glucose tolerance curve with the glucose-insulin tolerance curve provides an index of the sensitivity of the patient to insulin (Fig. 1). Sensitivity to insulin is indicated by the inhibition of alimentary hyperglycemia. On the basis of observations with this

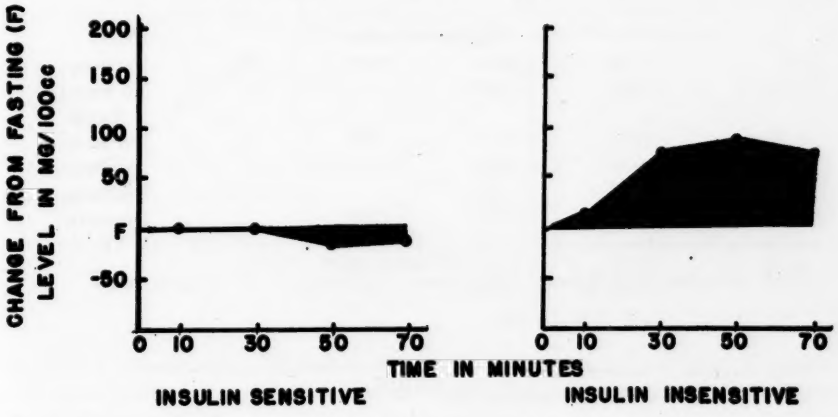


FIG. 2. Glucose-insulin area (shaded), which is expressed in the text in milligram-minutes.

TABLE 1. CALCULATED VALUES FOR THE GLUCOSE-INSULIN AREAS BEFORE AND DURING THE ADMINISTRATION OF DESOXYCORTICOSTERONE ACETATE AND AFTER AN INTERVAL WITHOUT TREATMENT

Patient	Glucose-insulin area in milligram-minutes: Before DOCA therapy	During DOCA therapy	After interval without treatment	Length of interval (weeks)	Change in sensitivity
		Insulin Insensitive			
E.T.	+5750	+1000	+3200	5	Increased
N.D.	+4075	−1350	+5225	1	Increased
R.M.	+4550	−5850	−2425	8	Increased
G.W.	+4575	+2950	—	—	Increased
R.D.	+2600	+350	+2450	8	Increased
C.M.	+4775	+25	+1500	8	Increased
G.M.(1)	+3775	−3250	+3500	3	Increased
(2)	+4575	+360	—	—	Increased
J.F.(1)	+5600	+2650	+4250	2	Increased
(2)	+4040	+1500	—	—	Increased
G.E.	+1225	+1425	+4125	12	No essential change
Mean	*+4140*	*−140*	*+2116*	—	—
S.D.	*±1231.8*	*±2436.2*	*±3032.6*	—	—
	t=4.95(p<0.01)				
Total number of patients, 9 Number with increased sensitivity, 8					$p<0.01$
		Insulin Sensitive			
S.T.	+650	+4350	−4300	12	Decrease
W.D.	−1250	−925	—	—	No essential change
W.H.	−2075	+1575	+1600	8	Decrease
H.W.	+50	+3950	—	—	Decrease
R.D.	−1000	+38	—	—	Decrease
R.A.	−1337	+375	+2200	2	Decrease
Mean	*−827*	*+1557*	*−166*	—	—
S.D.	*±910.5*	*±1752.6*	—	—	—
	t=2.45(p<0.05)				
Total number of patients, 6 Number with decreased sensitivity, 5					$p<0.01$

test, Himsworth (5b) classified diabetic patients into two groups, insulin-sensitive and insulin-insensitive. The degree of sensitivity to insulin may also be estimated by the size of the glucose-insulin area. This area, expressed in milligram-minutes, lies between the glucose-insulin tolerance curve and a horizontal line drawn from the fasting glucose level (Fig. 2). In the insulin-sensitive patient this area has either a negative or a slightly positive value, whereas in the insensitive patient it has a highly positive value.

In the present study, the change in size of the glucose-insulin area following the administration of desoxycorticosterone acetate was used as an index of altered sensitivity to insulin. In order to determine whether variation in alimentary absorption of glucose occurred, glucose tolerance curves were also obtained in 9 patients before and after the administration of desoxycorticosterone acetate.

After the estimation of insulin sensitivity, a dose of 10 mg. of desoxycorticosterone acetate was administered daily, intramuscularly, for ten days to each of 12 patients. In 2 other patients (R. McD. and D. W.) it was given for only four days. In 1 patient (J. F.) 5 mg. daily was given for ten days in one experiment, and 15 mg. daily for four days in another. On the last day of desoxycorticosterone administration, and again after an

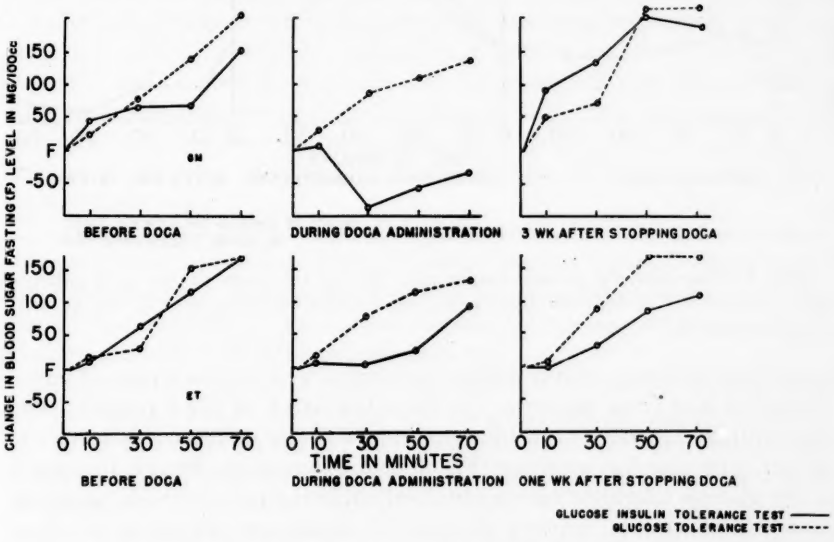


FIG. 3. Characteristic glucose-insulin and glucose tolerance curves in 2 patients with insulin-insensitive diabetes showing the effect of the administration of desoxycorticosterone acetate.

interval without treatment, the glucose-insulin tolerance tests were repeated.

RESULTS

The values for the glucose-insulin area before and during the administration of desoxycorticosterone, as well as after a period free of the steroid, are shown in Table 1. Of the 15 patients studied, 9 were initially insensitive to insulin and 6 were sensitive, judged by the standards of the test as outlined. An increase in sensitivity to insulin occurred in 8 of the 9 initially

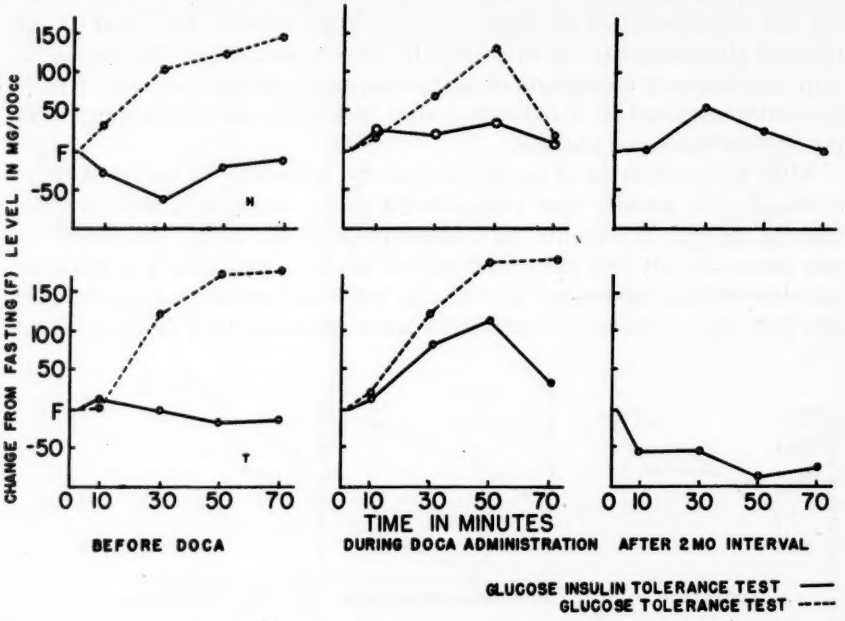


FIG. 4. Characteristic glucose-insulin and glucose tolerance curves in 2 patients with insulin-sensitive diabetes showing the effect of the administration of desoxycorticosterone acetate.

insensitive patients, with complete conversion to a sensitive type of curve (Fig. 3) in 6 of these patients. On the other hand, of the 6 patients who were initially sensitive to insulin, none showed any increase in sensitivity. In fact, 5 became less sensitive (Fig. 4) and 1 showed no change. Inasmuch as the glucose tolerance curves obtained while the patients were receiving desoxycorticosterone acetate showed no significant change from those obtained before the steroid was administered, the alteration in the glucose-insulin tolerance curve could not be ascribed to a change in alimentary glucose absorption (Figs. 3 and 4). The significance of the increase in sensitiv-

ity in the initially insensitive patients, and the decrease in the initially sensitive ones is shown by the value for p (6) and t (6) (Table 1). The analysis of the qualitative changes by the Chi-square method shows a significant value for p in the case of both the initially insensitive and the initially sensitive diabetic patients. The quantitative change in the glucose-insulin area in the insulin-insensitive group induced by the administration of desoxycorticosterone acetate likewise seems significant. The t value for the difference between the means is 4.95, $p<0.01$. In the case of the initially insulin-sensitive diabetic patients, however, the t value for the difference between the mean glucose-insulin area before and after the administration of desoxycorticosterone acetate is 2.45 $p<0.05$. This difference is just barely significant.

The state of altered sensitivity was found to be temporary in most of the group. Eight of the 9 initially insulin-insensitive patients were each retested after an interval which varied from one to twelve weeks following the period of desoxycorticosterone acetate administration. It was found that 7 of these patients had returned to a state of insensitivity, while 1 remained sensitive after eight weeks. Of the 6 initially sensitive patients, 3 were retested after an interval of two, eight and twelve weeks respectively. Of these, 2 patients were still insulin-insensitive and 1 appeared to have returned to his previous state of sensitivity.

A distinct change in insulin requirements was observed in only 1 patient in each group. One, initially insensitive to insulin, had required 35 units of insulin daily before the administration of desoxycorticosterone, whereas after receiving the steroid he required no insulin. One sensitive patient showed a rise in insulin requirement from 40 to 75 units daily. No consistent changes in the level of fasting blood sugar or in the degree of glycosuria were demonstrated.

Undesirable effects associated with the use of desoxycorticosterone were observed in only 1 patient (J. F.), in whom there developed edema of the lower extremities and dyspnea. A second period of administration of this steroid was carried out without occurrence of edema, employing intermittent administration of mercurial diuretics.

A small weight gain (3–5 pounds) occurred in 14 patients. One patient (D. W.) gained 11 pounds. There was no apparent relationship between the change in insulin-sensitivity with desoxycorticosterone and the amount of weight gain. The blood pressure was not significantly altered in any of the patients.

DISCUSSION

The evidence for the reliability of the glucose-insulin tolerance test as a measure of insulin-sensitivity has been presented by Himsworth and Kerr

(5). These authors also outlined the advantages of this procedure over the insulin tolerance test in the study of diabetic patients. The glucose-insulin tolerance test is based on the ability of intravenous insulin to suppress alimentary hyperglycemia. Theoretically, a change in results obtained with this procedure in any individual patient might be due either to a change in insulin sensitivity or to a change in the rate of glucose absorption. The factor of glucose absorption was eliminated in the present observations by the comparison of the glucose tolerance curves before and after the administration of desoxycorticosterone. Therefore, the change in responsiveness to insulin appears to be due to alterations in sensitivity.

The classification by Himsworth (5) of diabetic patients into insulin-sensitive and insulin-insensitive types, based on the results obtained with the glucose-insulin tolerance test, has been questioned by De Wesselow and Griffiths (7), who did not find a clear-cut distinction between the two groups. Our findings have been in agreement with Himsworth and Kerr. Furthermore, studies previously reported showed a high incidence of fatty metamorphosis of the liver in patients who were insulin-insensitive in contradistinction to patients who were insulin-sensitive. This also demonstrates an apparent fundamental difference between the patients who are sensitive to insulin and those who are not. The present observations of a difference in the effect of desoxycorticosterone on insulin sensitivity in the two groups likewise supports this concept.

The increase in sensitivity to insulin produced by the administration of desoxycorticosterone acetate to the insulin-insensitive diabetic may depend on a direct potentiation of the action of insulin, or may be mediated through its effect on factors which oppose the action of insulin. Cheng and Sayers (3) interpreted the increase in sensitivity to insulin in nondiabetic rats, produced by the administration of desoxycorticosterone acetate, to be due to the inhibition of the output of pituitary adrenocorticotropic hormone, and consequently of 11-oxysteroids by the adrenal cortex. If the effect of desoxycorticosterone were due to a direct potentiation of insulin activity, it should be qualitatively similar in all diabetics, with differences only in the degree of response. On the other hand, if desoxycorticosterone acetate increases sensitivity to insulin through the inhibition of insulin antagonists, its effect should be most apparent in patients in whom such factors play an important role.

Himsworth has summarized the evidence which suggests the presence of insulin antagonists, probably of pituitary origin, in insulin-insensitive diabetes (5). Our previous study of the correlation between the presence of fatty livers and insulin-insensitivity similarly led to the hypothesis that both were due to a common factor, possibly emanating from the anterior lobe of the hypophysis (1). The qualitative and quantitative differences in

the alterations in insulin-sensitivity in the two groups of diabetics in the present study suggest that 1) diabetes associated with insulin-insensitivity may be due to an excess of insulin antagonists possibly originating from the pituitary or adrenal glands, whereas diabetes which is insulin-sensitive may be due principally to lack of endogenous insulin, and 2) the administration of desoxycorticosterone acetate to the insulin-insensitive diabetic leads to the inhibition of insulin antagonists.

The decrease in sensitivity to insulin which was observed in the initally sensitive patients is difficult to interpret. In the small group of patients studied, 5 of the 6 showed an absolute decrease in insulin sensitivity. However, the statistical analysis of the degree of change indicates that this is barely significant. Although these observations require further investigation, a tentative interpretation of these paradoxical alterations appears to be warranted. Selye (8) has shown that large doses of desoxycorticosterone can produce a "stress" situation in animals. If the doses used in this study represent a relative excess for the initially insulin-sensitive patient, the resulting stress with the stimulation of the pituitary-adrenal axis may be responsible for the decrease in sensitivity.

The results of this study may explain the divergent reports in the literature of the effect of desoxycorticosterone in carbohydrate metabolism. Improved carbohydrate tolerance in humans after desoxycorticosterone acetate administration has been reported by Kohler and Fleckenstein (9) and McGavack and his co-workers (10). Winnett, Caldwell and Kahler (11) reported a transient decrease in insulin requirements in diabetic patients receiving deoxycorticosterone acetate. Patrono (12) reported no effect in 3 patients, an increase in carbohydrate tolerance in 1, and a decrease in another. In partially depancreatized rats, small doses of desoxycorticosterone acetate decrease glycosuria, whereas large doses increase glucose excretion (13). This observation is in keeping with the hypothesis that an excessive dose of desoxycorticosterone acetate (8) can produce stress and thus explain a "paradoxical" decrease in insulin-sensitivity. The reported patients (12) who showed no increase in sensitivity to insulin or improvement of glucose tolerance may have been initially sensitive, whereas those (9, 10, 11, 12) who did show this "anti-diabetogenic" action of desoxycorticosterone acetate may well have fallen into the initially insulin-insensitive group.

The failure to demonstrate a consistent decrease in insulin requirements, glycosuria or fasting blood sugar in these patients may have been due either to the short duration of the experiments or to some other unrecognized factors. Therefore no prediction of any possible value of desoxycorticosterone acetate, or similar steroids, in the management of insulin-insensitive diabetes can be made at this time.

SUMMARY AND CONCLUSIONS

Of 15 patients with diabetes mellitus who were studied by the glucose-insulin tolerance method of Himsworth, 9 were found to be insensitive to insulin and 6 sensitive. The administration of desoxycorticosterone acetate to these patients was followed in the initially insensitive diabetics, by a transient increase in sensitivity to insulin, whereas in the patients initially sensitive to insulin it produced either a decrease in sensitivity or no change.

REFERENCES

1. ZIMMERMAN, H. J.; MACMURRAY, F. G.; RAPPAPORT, H., and ALPERT, L. K.: Studies of the liver in diabetes mellitus. 2. The significance of fatty metamorphosis, *J. Lab. & Clin. Med.* **36**: 922, 1950.
2. CAMPBELL, J.: A method of assaying the potency of anterior pituitary extracts which increase liver fat, *Endocrinology* **23**: 692, 1938.
3. CHENG, C., and SAYERS, G.: Insulin hypersensitivity following the administration of desoxycorticosterone acetate, *Endocrinology* **44**: 400, 1949.
4. ZIMMERMAN, H. J.; PARRISH, A. E., and ALPERT, L. K.: Effect of desoxycorticosterone acetate on insulin sensitivity in diabetes mellitus, *Proc. Soc. Exper. Biol. & Med.* **73**: 81, 1950.
5. HIMSWORTH, H. P., and KERR, R. B.: (a) Insulin-sensitive and insulin-insensitive types of diabetes mellitus, *Clin. Sc.* **4**: 119, 1939. (b) Pituitary-like factors in blood and urine of diabetic patients and of animals treated with pituitary extracts, *Clin. Sc.* **4**: 287, 1940.
6. SNEDECOR, G. W.: Statistical Methods, ed. 4, Ames, Iowa, The Iowa State College Press, 1946, pp. 75–88.
7. DE WESSELOW, O. L. V., and GRIFFITHS, W. J.: Insulin resistance in diabetes mellitus and the effect of dietary carbohydrate, *Quart. J. Med.* **7**: 17, 1938.
8. SELYE, H.: Hypertension as a disease of adaptation, *Recent Progr. Hormone Research* **3**: 343, 1948.
9. KOHLER, V., and FLECKENSTEIN, A.: (a) Der Einfluss des synthetischen Nebennierenrindenhormons (Percorten) auf den diabetogenen Staubeffekt, *Klin. Wchnschr.* **20**: 844, 1941. (b) Die Stellung des Desoxycorticosteronacetat und Capillar-venose Blutzuckerdifferenz bei Normalen, *Deutsches Arch. f. klin. Med.* **189**: 530, 1942.
10. MCGAVACK, T. H.; CHARLTON, G. P., and KLOTZ, S.: Effect of desoxycorticosterone acetate on glucose tolerance in normal individuals and in patients with Addison's disease, *J. Clin. Endocrinol.* **1**: 824, 1941.
11. WINNETT, E. B.; CALDWELL, J. W., and KAHLER, J. E.: Transient anti-diabetogenic effect of synthetic adrenal cortical hormone: cortate, *J. Iowa M. Soc.* **30**: 45, 1940.
12. PARRONO, V.: Sugli effetti dell' impianto sottocutaneo di acetato di desossicorticosterone nel diabete mellito umano, *Il Progresso Medico* **4**: 619, 1948.
13. INGLE, D. J.: The effect of 11-desoxycorticosterone acetate upon the glycosuria of partially depancreatized rats, *Proc. Soc. Exper. Biol. & Med.* **69**: 329, 1948.

AN IMPROVED PELLET IMPLANTER

WILLIAM H. PERLOFF, M.D.

From the Endocrine Clinics of the Philadelphia General Hospital and the Medical Division of the Temple University Hospital and School of Medicine, Philadelphia, Pa.

THE use of pellets of compressed steroid crystals in the treatment of estrogen, androgen, and desoxycorticosterone deficiencies, is becoming increasingly popular. One implantation of one or more pellets is usually therapeutically effective, in selected patients, for from four to ten months.

Methods of implantation have varied, but most endocrinologists employ Kearns' pellet injector (1). Though extremely valuable, this instrument has three major drawbacks. The diameter of the orifice, into which the pellets are placed, is only slightly larger than the diameter of the pellets themselves, so that difficulty is experienced in their insertion. Fragmentation and loss of pellets are therefore common. Furthermore, it is not possible to place more than four, 75-mg. testosterone or desoxycorticosterone pellets end to end in this implanter and still leave an adequate distance between the last pellet and the skin incision. If, for example, six testosterone pellets are to be inserted, they must be placed in two separate channels. Finally, Kearns' instrument consists of three parts, which necessitates some manipulation of the trocar and plunger when more than four pellets are to be used.

In order to correct these three defects, a funnel has been placed at the upper end of the implanter so that the pellets may be dropped into it with ease, the shaft has been lengthened, and the trocar has been redesigned so that it may also be used as a plunger (Fig. 1).

The technique of implantation found most satisfactory by the author (2) is as follows: The patient lies on the table, face down, with arms about three inches from the body. A small area below the angle of the scapula towards the posterior axillary line is prepared with an antiseptic, and infiltrated with novocain and 1 per cent adrenalin. About 1–2 ml. of solution is injected anteriorly along the projected course of the implanter. The skin is pinched and raised between the thumb and index finger of one hand, and a thin #11 Bard-Parker blade inserted to its base into this fold. The incision should penetrate below the skin into the subcutaneous tissue and should be large enough to allow easy entrance of the implanter.

The implanter,[1] with trocar in place, is then directed along the line of

Received for publication January 20, 1951.

[1] Manufactured by George P. Pilling & Son Company, 3451 Walnut Street, Philadelphia 4, Pa.

novocain infiltration as far as is required to introduce the requisite number of pellets, and still leave a distance of at least one-half inch from the end of the last pellet to the skin incision (Fig. 1). The trocar is withdrawn and held between the index and middle fingers at the base, which allows both hands to be free for further manipulation. The pellets, as required, are dropped into the funnel, and the trocar is employed, if necessary, to direct them. The trocar is then reinserted into the cannula, and, with the least pressure that is required to hold the pellets in place, the cannula is withdrawn from around them. The entire instrument is removed and the

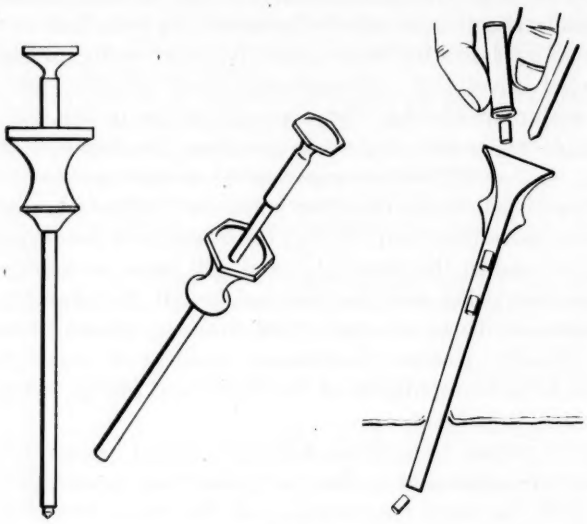


FIGURE 1

skin incision closed under slight tension with sterile adhesive tape or adhesive bandage. The dressing is removed in approximately four days. The purpose of this procedure is to leave the pellets in the loose tissue of the posterior axillary fold where they are not subject to trauma.

In well over 250 implantations,[2] there have been no major complications. Occasionally, when too much anesthetic solution has been employed, the patient has complained of slight adrenalin effect. This is particularly true when pituitary or adrenal insufficiency is present. The occasional bleeding is slight and is easily controlled by temporary pressure. The procedure entails no discomfort, and no pellets have been extruded.

[2] Using 25-mg. pellets of compressed estradiol (Progynon), 75-mg. pellets of compressed testosterone (Oreton-F), and 75-mg. pellets of compressed desoxycorticosterone acetate (Cortate).

Criteria for pellet implantation must be carefully observed. No patient, for example, with uterus intact should be given an estradiol pellet (2). Patients, who are suitable for pellet implantation, are extremely grateful for this type of therapy, being relieved of the necessity of receiving repeated injections, or of giving thought to oral medication. Furthermore, the constant release of small amounts of hormone from the pellets appears to be more effective, in many patients, than any other type of therapy.

If the pellets appear to be exhausted within four months of implantation, heat applied to the pellet site will often reestablish their effectiveness. The reason for this appears to be too dense encapsulation of the pellets, a condition which may be expected to occur in some few patients.

SUMMARY

1. An improved pellet implanter is described which allows the subcutaneous insertion of the requisite number of estradiol, testosterone, or desoxycorticosterone pellets end to end, with one manipulation, in from five to ten minutes.

2. The implantation site of choice is in the posterior axillary line below the angle of the scapula, where the opportunity for trauma is minimal. As many as six successive implantations have been made on the same side in one patient in the course of thirty-six months.

3. The ease of pellet implantation and the effectiveness of this type of therapy in selected patients strongly recommend its increased use in clinical practice.

Acknowledgment

The author wishes to express his appreciation to Mr. James McBrinn of Pilling Company for his valuable suggestions.

REFERENCES

1. Kearns, W.: Pellet implantation of hormones in urology, *J. Urol.* **47**: 587–590, 1942.
2. Perloff, W. H.: Treatment of the menopause, *Am. J. Obst. & Gynec.* **58**: 684–694, 1949.

CRETINISM WITH GOITER: A CASE REPORT*

JOHN B. STANBURY, M.D.

From the Thyroid Clinic of the Massachusetts General Hospital, Boston, Mass.

CRETINOUS patients with goiter may show a high accumulation of radioactive iodine. In studies on 3 such siblings (1) the radioactive iodine was found to be rapidly discharged from the thyroid upon the administration of potassium thiocyanate, an observation which was interpreted to mean that the thyroid glands of these patients were unable to incorporate iodide into a protein complex. This case report concerns another goitrous cretin, referred by Dr. C. D. Cook of the Children's Hospital of Boston.

Shirley O., MGH #696322 a 13-year-old female, was said to have had a large head and an umbilical hernia at birth. At five months of age because of failure to gain, poor feeding, drowsiness and constipation, a diagnosis of cretinism was made. She was given desiccated thyroid in dosages varying up to 2 grains daily. Prompt improvement followed, but she did not walk until she was 2 and began to talk only when she reached the age of 3. Mental retardation was always evident. One year before admission her parents noticed an enlargement in the region of her thyroid which seemed to increase slowly in size. Thyroid medication was withheld for one month before hospital admission.

There was a history of undefined thyroid disease in the maternal grandmother and a maternal aunt. A brother aged 4 was normal. The family and past histories were otherwise normal.

The patient was a jovial well developed female. Her hair was dark brown, her skin cool, rough and dry. Her nose was short and had a broad base. Her speech was slow, thick and indistinct. Her voice was husky. Her tongue was larger than normal. The thyroid was enlarged to about four times normal size and was multinodular. A bruit was present. There was no umbilical hernia. Her weight was 46.8 Kg. and her height 149.7 cm.

Urinalysis yielded normal results. The white blood count was 10,400 cells per cubic millimeter, with 59 per cent polymorphonuclear cells. The value for hemoglobin was 13.5 grams per 100 cc. Two basal metabolic rate determinations were −30 and −37 per cent. The blood cholesterol was 277 and 327 milligrams per cent on successive determinations. The serum protein-bound iodine content was 0.4 and 0.4 microgram per 100 cc. on successive analyses. Among the observations made at the Children's Hospital shortly before the patient was referred to us for study were an I.Q. of 50 and a bone age approximately equal to chronologic age.

Radioactive iodine study: On the fourth hospital day the patient was given 100 microcuries of I^{131}. As shown in Figure 1 this accumulated rapidly in the gland as meas-

Received for publication January 19, 1951.

* Supported by grants from Parke, Davis and Company, Detroit and the "H. N. C." gift of Harvard University.

ured by a shielded Geiger-Müller tube placed 24 cm. above the gland. When a plateau had been reached several hours later, 2 grams of potassium thiocyanate were administered by mouth. Shortly thereafter there was a rapid disappearance of the radioactive iodine from the region of the thyroid gland.

Four months later the patient was readmitted to the hospital for thyroidectomy. She had received no thyroid medication in the interim. Physical findings were not appreciably changed except that the thyroid seemed larger. Her basal metabolic rate was −41 per cent. The surgical procedures and the postoperative courses were uneventful. She is being maintained in a satisfactory state on 0.065 Gm. of thyroid daily.

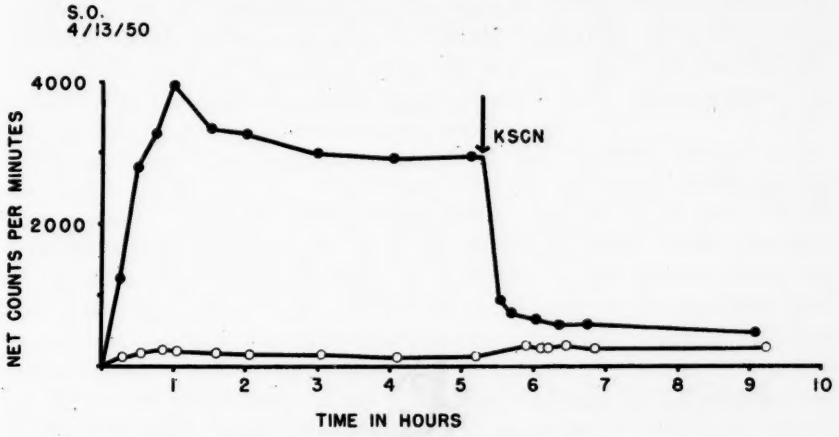


FIG. 1. Radioactive iodine accumulation in the thyroid of a 13-year-old goitrous cretin. Ordinate: net counts per minute. Abscissa: time in hours. At zero time, 100 microcuries of radioactive iodine were administered orally. The closed circles are counts per minute registered over the thyroid. The open circles are counts per minute registered over the thigh. At the arrow, 2 grams of potassium thiocyanate were administered orally.

The histology of the removed gland was studied by Dr. Austin Vickery, who reported as follows: The gland is composed of adenomas of varying degrees of cellular differentiation. Some are composed of solid masses of large thyroid cells arranged in packed clusters and cords and others of smaller cells showing evidence of acinar formation with colloid production. Focal areas of the latter exhibit hyperplasia, with papillary infoldings of follicle walls, columnar cells and a scalloping of the acinar colloid. Occasional adenomas appear to be in transitional phases from inactive solid masses of embryonal cells to attempts at functional hyperplasia. Most of the adenomas are not only differentiated into adult acini but the majority of the latter exhibit evidences of hyperplasia. The degree of hyperplasia in many areas is intense and exaggerated, and in certain areas, is suggestive of the atypicality of malignant neoplasia.

The excised gland contained 0.62 microgram of total iodine per gram of wet tissue, and 0.07 microgram of precipitable iodine per gram of wet tissue.

In this patient, as in those previously reported, there seemed to be no difficulty in the accumulation of iodide by the thyroid. Indeed the ac-

cumulation proceeded in a uniformly rapid fashion. The rapid discharge of this iodide by KSCN again suggests that the mechanism involved in this form of cretinism is an inability to combine iodide into a protein complex. Intense compensatory hypertrophy of the gland results.

REFERENCE

1. Stanbury, John B., and Hedge, Alice N.: A study of a family of goitrous cretins, *J. Clin. Endocrinol.* **10**: 1471, 1950.

Letter to the Editor

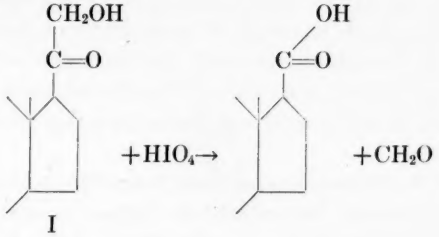
THE CHEMICAL ASSAY OF URINARY CORTICOSTEROIDS

To the Editor:

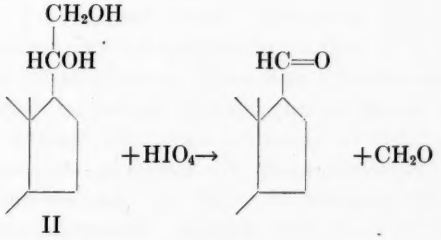
There is an increasing interest in the assay of urinary corticosteroids by methods based on the generation of formaldehyde from these compounds by periodic acid. In view of statements made in two articles recently published in this journal it seems appropriate to make some comment on the reaction of adrenal steroids with periodic acid.

The authors of one paper (1) state, " . . . the number of moles of formaldehyde yielded varies, depending upon the side-chain configuration of the unknown steroid." The authors of the other paper (2) state, "With periodic treatment each molecule of desoxycorticosteroid yields one mole of formaldehyde; for every mole of 17-hydroxycorticoid two moles of formaldehyde result." Use of the terms "desoxycorticosteroid" and "17-hydroxycorticoid" is unfortunate in this connection since the reaction of periodic acid with adrenal compounds to produce formaldehyde depends on the structural features of the side chain and not on the presence or absence of oxygen atoms elsewhere in the molecule.

In order to produce formaldehyde the side chain must have one of the following structures (I or II):

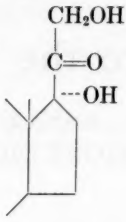


I



II

The presence of a 17-hydroxyl group, as in cortisone,



does not contribute to the yield of formaldehyde. Only a terminal carbon atom which bears an alcohol group and which is adjacent to a carbon atom which bears a ketone group (I) or which bears another alcohol group (II) can yield a molecule of formaldehyde in this reaction.

Daughaday, Jaffe and Williams (3), in the description of their method for "Chemical Assay for 'Cortin,' " also write, "Fieser, Fields and Lieberman (4) have stated that compounds with a ketol side chain will yield one mole of formaldehyde for each mole of steroid. With a dihydroxyacetone type of side chain the reaction under certain conditions proceeds to form between one and two moles of formaldehyde. With the glycerol type of side chain there is a greater likelihood of the removal of two carbon atoms." Apparently Daughaday and co-workers and the authors of the more recent papers have confused the consumption of periodic acid with production of formaldehyde, for nowhere in their paper do Fieser and co-workers discuss the yield of formaldehyde; indeed, formaldehyde is not even mentioned. No matter how much periodic acid may be consumed only one molecule of formaldehyde can be produced by the structures discussed under the conditions used. Removal of carbon atoms does not necessarily mean production of formaldehyde. Only the terminal $-CH_2OH$ can yield formaldehyde when cleaved from the corticosteroid. Jackson (5) has given a full discussion of periodic acid oxidation and the products formed by such oxidation.

Because of the 1:1 relationship between formaldehyde and corticosteroid it is justifiable to convert formaldehyde values to corticosteroid values even though it is possible that a small portion of the formaldehyde may arise from nonsteroid precursors. Since the urinary "corticosteroids" consist essentially of a mixture of cortical hormones and their metabolic products, some uncertainty necessarily is introduced by adoption of a conversion factor based on any specific molecular weight. However, it would seem reasonable to assume a molecular weight corresponding to that of the $C_{21}O_4$ corticosteroids (for example, corticosterone, mol. wt. 346), the molecular weights of which are approximately intermediate between those of the $C_{21}O_3$ (for example, desoxycorticosterone, mol. wt.

328) and the $C_{21}O_5$ (for example, cortisone, mol. wt. 360) corticosteroids. The molecular weight of a formaldehydogenic metabolic product of an adrenal hormone in all likelihood would not differ from that of the hormone by more than 6 to 8 units. Assuming a molecular weight of 346 the conversion would be

$$\text{mg. formaldehyde} \times 11.5 = \text{mg. corticosteroids.}$$

HAROLD L. MASON, PH.D.

Division of Biochemistry,
Mayo Clinic,
Rochester, Minnesota

REFERENCES

1. LLOYD, C. W., and LOBOTSKY, JULIA: Studies of urinary corticosteroid by a method permitting analysis of steroids poorly soluble in water. I. Normal adrenal function, *J. Clin. Endocrinol.* **10:** 1559–1569 (Dec.) 1950.
2. HIOCO, DESYR, and SAMTER, MAX: The excretion of 17-ketosteroids, 11-oxycorticosteroids and "11-desoxycorticosteroids" in patients with bronchial asthma, *J. Clin. Endocrinol.* **10:** 1570–1578 (Dec.) 1950.
3. DAUGHADAY, W. H.; JAFFE, H., and WILLIAMS, R. H.: Chemical assay for "cortin." Determination of formaldehyde liberated on oxidation with periodic acid, *J. Clin. Endocrinol.* **8:** 166–174 (Feb.) 1948.
4. FIESER, L. F.; FIELDS, MELVIN, and LIEBERMAN, SEYMOUR: Concerning the characterization of possible cortical hormone metabolites in urine, *J. Biol. Chem.* **156:** 191–201 (Nov.) 1944.
5. JACKSON, E. L.: Periodic acid oxidation, *in* Adams, Roger: Organic Reactions. New York, John Wiley & Sons, Inc., 1944, vol. 2, pp. 341–375.

The Association for the Study of Internal Secretions

ABSTRACTS OF PAPERS PRESENTED AT THE THIRTY-THIRD ANNUAL MEETING

THURSDAY, FRIDAY AND SATURDAY,
JUNE 7, 8, AND 9, 1951

Chalfonte-Haddon Hall
Atlantic City, New Jersey

(Abstracts arranged according to numbers on printed program)

THURSDAY, JUNE 7, 1951

9:30 A.M.—GENERAL SUBJECTS

DRS. C. N. H. LONG AND LAWSON WILKINS, PRESIDING

1. Endocrine Studies on Inherited Obesity in the Mouse.
Robert S. Speirs (introduced by Dr. R. K. Meyer).

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

Approximately two years ago, it was observed that some of the mice in certain genetic stocks at the Roscoe B. Jackson Memorial Laboratory were becoming excessively obese. These animals showed extreme fat deposition in the trunk region and attained a weight of over 70 grams. Some weighed as much as 100 grams. The cause of this abnormality was traced back to a recessive mutation in two "V" stock animals (Ingalls, Dickie and Snell, 1950).

The endocrine studies to date on these mice can be summarized as follows:

A. Hypophysis—Removal of the pituitary prevented the obesity from progressing in all of the 13 mice operated upon.
B. Adrenal—Removal of the adrenal gland did not have an immediate effect on the rate of growth or fat deposition of 5 obese mice. However, records maintained over an 8-month period indicated that these animals did not gain as much weight as did the obese mice not operated upon.
C. Thyroid—Four obese mice fed powdered thyroid (1 mg. per gram of food) showed a slight loss of weight, followed by a rapid growth when the hormone was removed from the diet.
D. Gonads—The obese mice did not mate. The ovaries were immature in appearance and the testes undescended.

Growth curves and other data pertaining to these mice are presented and discussed.

2. Endocrinologic Effects of Amphenone "B."

Roy Hertz, Milton J. Allen (by invitation) and *Wm. W. Tullner* (by invitation).

National Cancer Institute, Bethesda, Maryland.

Amphenone "B" (1,2-bis-(p-aminophenyl)-2-methylpropanone-1 dihydrochloride) (Allen & Corwin, *J.A.C.S.* **72:** 117, 1950) exerts a progestational effect upon the endometrium of the Clauberg rabbit, its progestational potency being equivalent to 0.5% that of progesterone. In the ovariectomized rat it exerts an atypical folliculoid effect on the uterus and vagina when fed at 0.2 mg./Gm. of body weight daily. At this dose it increases the adrenal weight threefold in five days. These enlarged adrenals are of a light yellow color and retain normal zonal and cytologic relationships. The thyroid increases fourfold after fifteen days of treatment and is ten times normal size after ninety days of treatment. The effects upon adrenal and thyroid are absent in the hypophysectomized animal, suggesting that both these responses are brought about through a pituitary mechanism similar to that by which thiouracil induces thyroid hypertrophy. The relatively limited thymic involution accompanying the marked adrenal enlargement indicates that the corticoid output from these large adrenals is quite low.

3. Effect of Vitamin A and Compound L on the Thyroid, Adrenal and Lymphoid Tissue of the Rat.

William L. Money, Jean Fager (by invitation), *Virginia Lucas* (by invitation) and *Rulon W. Rawson.*

The Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Twelve groups of immature male rats were maintained on a vitamin A-free diet for five and one-half weeks. Half of the animals received 0, 2, 10, 25, and 50 I.U. of vitamin A daily (oral administration) and injections of compound L (10 mg./100 Gm. body weight). The other groups received only the above doses of vitamin A. At autopsy, weights of the thyroid, adrenal, thymus and lymph nodes were determined. I^{131} collected by the thyroid gland was also calculated.

The results indicate that both compounds decrease the concentration of radioactive iodine found in the thyroid. Compound L, however, produces a more marked depression than high doses of vitamin A. Low or high intakes of vitamin A apparently increase adrenal weight. Compound L was found to increase adrenal weight with the higher levels of vitamin A. Increased thymus weight was observed with increased intake of vitamin A. Compound L not only prevents this but decreases thymus size. There appears to be a direct correlation between vitamin A intake and lymph node size. Compound L produces marked hypertrophy of lymph nodes except with the higher levels of vitamin A. The significance of these observations is considered.

4. Tracer Studies with Radioactively Labeled Prolactin Preparations.

Martin Sonenberg (by invitation), *William L. Money, Albert S. Keston* (by invitation), *Patrick Fitzgerald* (by invitation) and *John T. Godwin* (by invitation).

Memorial Cancer Center and New York University-Bellevue Medical Center, New York, N. Y.

Prolactin preparations labeled by iodination with I^{131} were administered intracardially to normal female and male Sprague-Dawley rats. Several organs were assayed for radioactivity following injection. The concentration of radioactivity in the ovaries increased rapidly until a maximum was reached between twenty and thirty minutes after injection.

Subsequently, the concentration of radioactivity fell with a biologic half-life of approximately one and one-half hours. Radioautographic studies demonstrated radioactivity in the ovaries, the radioactivity being concentrated in the corpora lutea with essentially no activity in the ovarian follicles. In the female rat no significant localization of radioactivity was detectable in the mammary tissue of normal adult, immature, pregnant, or lactating animals. Control studies with I^{131} labeled iodine and I^{131} labeled serum albumin revealed significantly less radioactivity in the ovaries, at all times examined. By radioautography, radioactivity was demonstrable in the ovarian follicular fluid following the administration of I^{131} labeled serum albumin. Assay of the I^{131} labeled prolactin preparations revealed no significant reduction in biologic activity of a preparation, which was labeled with an iodine to protein molecular ratio of 1.6.

5. Effect of Prolactin on Phosphorus Metabolism of Pigeon Crop-Sac: Applicability to Bioassay of Prolactin.

Robert W. Brown (by invitation) and *George Sayers.*

Departments of Pharmacology and Obstetrics and Gynecology, University of Utah College of Medicine, Salt Lake City, Utah.

This investigation was undertaken to study the mechanism of action of prolactin on the pigeon crop-sac and to devise an assay superior to current methods. Prolactin-induced growth of the crop-sac is associated with an increased synthesis of phosphorus-containing compounds. The concentration of P^{31} and the turnover of P^{32} in the crop-sac are both useful indices of prolactin activity.

Prolactin was administered intramuscularly to immature pigeons once daily for four days. Twenty-four hours after the last injection, P^{32} was injected intraperitoneally and the birds were sacrificed eight hours later. Total P^{31} and P^{32} concentrations and P^{31} and P^{32} concentrations of various phosphate fractions were determined.

Under the influence of prolactin, inorganic phosphorus enters the cells of the crop-sac at an increased rate and apparently serves as the sources of phosphate for the synthesis of phosphorylated intermediates and nucleic acids.

The following total doses of prolactin induced significant increases in the concentration of P^{31} per Kg. of crop-sac wet tissue: no prolactin (controls), 62 mM; 10 micrograms, 70 mM; 100 micrograms, 73 mM; 250 micrograms, 88 mM; 500 micrograms, 87 mM; and 1000 micrograms, 97 mM P^{31}.

6. The Clinical Effect of Diethylstilbestrol on Osteoarthritis in Women.

Alton C. Grorud.

Department of Internal Medicine, The South Bend Clinic, South Bend, Indiana.

One hundred women, ranging in age from 19 to 70 years, presenting significant signs and symptoms of osteoarthritis, were treated orally with diethylstilbestrol in the dosage of 0.5 mg. twice daily for a total duration of three weeks. The effects were observed clinically for a period of approximately one year thereafter in each case. Forty-six were relieved of pain to constitute temporary remissions; 35 experienced definite but not complete amelioration; 19 derived no beneficial effect. In those obtaining favorable results, pain relief was usually noted within five days after starting treatment; they remained comfortable for an average of eight and one-half weeks. The results in all age groups were essentially constant.

There is evidence that osteoarthritis is primarily a metabolic disorder having strong

endocrine implications in its pathogenesis. The interrelationships of the estrogens and the other steroids possessing the cyclopentanophenanthrene nucleus, together with the modifying effects of certain vitamins and enzyme systems acting at cellular levels must be clarified before the etiology and treatment of osteoarthritis become rational. The fact that mitochondria, containing the fundamental intracellular enzyme oxidation factors, are not present in the cartilage of joints may be significant.

7. A Syndrome, Distinct from Acromegaly, Characterized by Spontaneous Lactation, Amenorrhea, and Low Follicle-Stimulating Hormone Excretion.

Anne P. Forbes, Philip H. Henneman (by invitation), *Grace C. Griswold* (by invitation) and *Fuller Albright.*

Department of Medicine, Harvard Medical School and the Massachusetts General Hospital, Boston, Mass.

We have followed for nine years a nulliparous young woman who complained of spontaneous lactation and acquired amenorrhea. She presented moderate obesity, seborrhea, slight hirsutism, a mottled blush over face and neck, and a persistent milky discharge from both breasts. The excretion of follicle-stimulating hormone has been consistently low, whereas other tests of anterior pituitary function, including an insulin tolerance test and 17-ketosteroid excretion, have shown normal results. Absence of estrin was indicated by failure of menstruation following a course of progesterone. Temporary inhibition of lactation has been obtained with intermittent estrin therapy. Acromegaly, which otherwise would fit these findings, was ruled out by the patient's appearance and the absence of an elevated inorganic serum phosphorus level. Arrhenoblastoma was ruled out by laparotomy. Seven more women with similar findings have been studied.

The low excretion of follicle-stimulating hormone in these patients is in contrast to the normal or elevated levels observed two to thirty-two weeks post partum in 15 normal women, 9 of whom were nursing. Eight of these 9 were amenorrheic.

8. Studies on a Patient with Diabetes Insipidus and Adrenocortical Insufficiency.

Herbert Gershberg, Stephen Leslie, and *Elaine P. Ralli.*

Department of Medicine, College of Medicine, New York University-Bellevue Medical Center, New York, N. Y.

A 42-year-old male has been studied since October 1947 when he first complained of impotence and polyuria, and was voiding 6 liters of urine daily, with a specific gravity of 1.001. The Hickey and Hare test indicated diabetes insipidus. The urine output was controlled with pitressin. In April 1949 he complained of severe weakness; the serum sodium level was 124 mEq./L. and the chloride, 96 mEq./L. A diagnosis of adrenal cortical insufficiency was made and he was treated with DOCA, cortical extract and salt. In spite of this he suffered 3 typical attacks of adrenal insufficiency. Laboratory studies showed:

Eosinophil counts	
Resting	500–600/cu. mm.
Response to—	
ACTH	18% decrease
Epinephrine	29% decrease

Ketosteroid excretion	
Control	5.8 mg./24 hours
After 48 hours of ACTH	7.3 mg./24 hours
Corticosteroid excretion	
Control	0.3 mg./24 hours
After 48 hours of ACTH	0.96 mg./24 hours
Basal metabolic rates	−14% and −32%

Renal function studies made while the patient was taking oral cortisone (25 mg. four times daily) showed an elevated G.F.R. and a depressed Tm paH. Antidiuretic assays of urine showed no A.D.S. in the control sample but a positive antidiuretic result after pitressin and after ACTH.

This appears to be the first case in which diabetes insipidus and adrenocortical insufficiency occurred in the same patient.

9. The Eosinophil Response to Stress of Patients With Surgically Produced Thalamic and Hypothalamic Lesions.

William H. Perloff, Lester M. Levy (by invitation) and *Agamemnon Despopoulos* (by invitation).

The Endocrine Clinics, Philadelphia General Hospital, Philadelphia, Pa.

Since 1949, 15 schizophrenic patients have had bilateral lesions produced in the thalamus and/or hypothalamus for the treatment of their psychoses by Drs. Spiegel and Wycis, employing their modification of the Horsley-Clarke sterotaxic apparatus. Patients were studied endocrinologically before and at various intervals after surgery. Four showed altered responses to certain procedures. Their lesions were distributed between the dorsomedial and the anterior nuclei of the thalamus and the posterolateral hypothalamus. In all 4 patients, eosinophil responses to 0.3 mg. of epinephrine, 25 mg. of ACTH, and 0.1 unit/Kg of insulin were normal preoperatively, and were usually normal for the first month postoperatively. However, after this time, responses to epinephrine were almost invariably abnormal. Insufficient eosinophil fall was similarly but less frequently encountered with insulin and ACTH. Two patients are presented in detail. In 1 patient, following a second operation, a marked sustained rise in fasting eosinophil levels to four times the preoperative values was noted. This patient also showed evidence of increased insulin sensitivity as measured by insulin tolerance tests.

10. Steroidal Control of Cerebral Metabolism in Man.

R. C. Bentinck (by invitation), *G. S. Gordan, J. E. Adams* (by invitation), and *T. B. Leake* (by invitation).

University of California School of Medicine, San Francisco, California.

The striking mental manifestations with often accompany administration of steroids have stimulated the authors to study the effects of deficiencies of such compounds on human cerebral metabolism. The Kety technique for quantitation of cerebral blood flow, and simultaneous determinations of arteriovenous differences of oxygen, carbon dioxide, and sugar have enabled us to study the metabolism of these substances by human brain *in vivo*.

We have compared the cerebral metabolic rates of normal human subjects with those of patients with hypopituitarism and pre-adolescent eunuchoidism of testicular origin.

Hypopituitary subjects consistently show a slow rate of cerebral metabolism. In

contrast, there is a tendency for patients with pre-adolescent eunuchoidism (hypoleydigism) to show an increased rate of cerebral metabolism, similar to that of the castrate immature rat (*Science* **109:** 337, 1949).

Since the sluggish cerebral metabolic rate of pituitary deficiency is not similarly produced by a deficiency of testosterone, another factor must be responsible. This may be adrenocortical, since Abood and Kocsis (*Proc. Soc. Exper. Biol. & Med.* **75:** 55, 1950) have demonstrated that the decreased rate of anaerobic glycolysis of the brain of hypophysectomized rats is increased by ACTH. We therefore studied the action of a potent water-soluble adrenocortical steroid, desoxycorticosterone glucoside. This compound restored to normal the decreased cerebral metabolic rate of hypopituitarism and reduced to normal the increased cerebral metabolic rate of eunuchoidism.

These studies suggest that testosterone normally exerts a restraining influence upon cerebral metabolism, while certain adrenocortical steroids may have a contrary action. No implications relating to mental function or emotional state are suggested by these studies.

11. Dual Control of ACTH Release.

Claude Fortier (introduced by Hans Selye).

Institut de Médecine et de Chirurgie Expérimentales, Université de Montréal, Montreal, Canada.

The ACTH response to systemic (epinephrine, cold, histamine) and to emotional (immobilization) and sensory (sound) stimuli was studied in hypophysectomized rats, bearers of intraocular, homologous transplants of the adenohypophysis. These animals, along with normal controls, were stimulated at weekly intervals, and the fall in circulating blood eosinophils used as an index of ACTH release. Epinephrine, cold and histamine brought about a definite eosinopenia in both normal and grafted animals. Sound and immobilization induced a marked fall of the eosinophils in the intact, but none in the grafted animals. These results suggest a dual regulation of ACTH release: one purely humoral, in response to systemic stimuli; the other, probably neurohumoral, mediated by the hypothalamo-hypophyseal neurovascular pathways, and coming into play under the influence of nervous or emotional stimuli.

12. Hormonal Regulation of Phagocytosis.

Paola S. Timiras (introduced by Hans Selye).

Institut de Médecine et de Chirurgie Expérimentales, Université de Montréal, Montreal, Canada.

The role of the anterior pituitary and of the adrenal cortex in the regulation of phagocytosis was studied in normal and stressed animals.

Exposure to stress stimulates the phagocytic activity of the reticulo-endothelial system in intact, but not in adrenalectomized or hypophysectomized animals. In the latter, administration of cortical extract, and to a lesser extent of adrenocorticotropic hormone (ACTH) and of somatotropic hormone (STH) restores the capacity of the reticulo-endothelial system to increase its phagocytic activity under stressing conditions.

Our results suggest that the anterior hypophysis and the adrenal cortex exert a regulatory influence upon phagocytosis. This is especially evident in conditions of stress. Such regulation has an important bearing upon the interpretation of the mechanisms by which the pituitary-adrenal function increases the resistance of the organism to pathogenic agents.

13. Metabolic and Endocrine Changes With Hypoglycemia.

H. P. Taft, Max Miller, William R. Drucker, and *James W. Craig* (introduced by Hans Hirschmann).

Western Reserve University, Cleveland, Ohio.

Epinephrine has long been held to play a significant role in recovery from hypoglycemia. More recently the adrenal cortex has been invoked. In an examination of these factors one-hour infusions of glucose (1 gram per kilogram) were given to normal subjects. During the recovery from the ensuing secondary hypoglycemia, and related to its degree, a rise in blood pyruvic acid was noted. Prolongation of the hyperglycemia by three-hour glucose infusion delayed the onset of hypoglycemia and its associated pyruvic-acid rise; a rise in plasma citric acid was also observed. There was a significant fall in eosinophil count, chronologically related to the hypoglycemia. In assessing the role of epinephrine and ACTH on the changes seen in these intermediaries during recovery from hypoglycemia, it was found that epinephrine produced similar changes but ACTH did not. It is suggested therefore that the changes following this form of hypoglycemia could be due, in part at least, to the direct metabolic action of endogenously produced epinephrine rather than an effect mediated through the pituitary-adrenal axis. A comparison of the effects of insulin-induced hypoglycemia is also presented.

14. Inhibition of the Gametokinetic Response in Male "Rana Pipiens" With Adrenolytic Drugs.

Sarah L. Clark (by invitation), *Robert B. Greenblatt,* and *Robert M. West* (by invitation).

Medical College of Georgia, Augusta, Ga.

In rather extensive studies on over 500 frogs we have found that several representative adrenolytic drugs will block the release of spermatozoa by the adult male frog, *Rana pipiens,* if injected in the dorsal lymph sac prior (30 seconds–1 minute) to the injection of chorionic gonadotropin, pituitary gonadotropin, human pregnancy urine, levo-epinephrine, levo-arterenol and epinephrine hydrochloride.

The optimal dose of adrenolytic agent necessary for inhibition of emission of spermatozoa was found to be: Benodaine Hydrochloride (Merck)—2.5 mg.; Dibenamine hydrochloride (Smith, Kline & French)—10.0 mg. to 12.5 mg.; Compound C.C.K. 179 (Sandoz)—2.0 to 4.0 mg.; Priscoline Hydrochloride (Ciba)—10.0 mg.; Compound S.K.F. #688-A (Smith, Kline & French)—3.0 mg. Studies thus far suggest that Benodaine Hydrochloride is the most effective agent in inhibiting release of spermatozoa in the male frog.

Doses of the control gonadotropic substances were as follows: 50 I.U. of chorionic gonadotropin, 1 R.U. of pituitary gonadotropin, and 1 to 5 cc. of unconcentrated human pregnancy urine. These dosages were above the minimal dose level required to produce a positive pregnancy test in *Rana pipiens.*

The approximate minimal dose of levo-epinephrine (Sterling-Winthrop) producing gametokinesis was 0.005 mg. and of levo-arterenol (Sterling-Winthrop), 0.05 mg. Doses of epinephrine hydrochloride (Adrenalin, Parke Davis) injected varied from 0.01 to 0.02 mg., as smaller quantities did not produce a uniformly positive response (gametokinesis).

Evidence obtained strongly suggests that the emission of spermatoza is mediated by the epinephrine activation of an adrenotropic receptor in the sperm ejaculatory apparatus

of the male frog. The possible role of the pituitary in provoking endogenous epinephrine release and subsequent emission of spermatozoa, upon the injection of pituitary and chorionic gonadotropins, has not been established. *Rana pipiens* promises to be a good test animal for the bioassay of epinephrine by standardizing 1) the dose of epinephrine necessary for gametokinesis and 2) the dose of adrenolytic drug necessary to inhibit epinephrine-induced gametokinesis.

2:30 P.M.—PITUITARY AND ADRENAL GLANDS

DRS. J. S. L. BROWNE AND JANE RUSSELL, PRESIDING

15. Effect of Hypophyseal Growth Hormone (GH) on the Thiocyanate Space and Muscle Electrolytes.

Adrienne Applegarth Batts (by invitation) and *Leslie L. Bennett.*

Division of Physiology and Institute of Experimental Biology, University of California, Berkeley, California.

Thirteen groups of 3 to 8 rats per group were studied. There were three groups of hypophysectomized controls, three groups of hypophysectomized rats treated with 0.5 to 1.0 mg. of GH per day, five groups of normal controls, and two groups of normal rats treated with 1.0 mg. of GH per day. The GH preparation was free from antidiuretic activity by the Ham and Landis test at a single dose of 0.5 mg. (the maximum single dose used in these experiments). Determinations of muscle and serum Na and K, and thiocyanate space of total body and muscle were made. GH treatment increased significantly the total body thiocyanate space over that of the hypophysectomized and normal controls. The muscle thiocyanate space was similarly increased when determined. Hypophysectomy produced a fall in the concentration of muscle K in the three groups (range 0.3 to 9.4 mEq./Kg., wet weight). This fall was statistically significant ($p = .02$) in one group. The administration of GH restored the muscle potassium to or towards normal in all groups of hypophysectomized rats and the change was statistically significant ($p = .05$) in one. There was a trend for the muscle sodium to be elevated after hypophysectomy and to be lowered by GH treatment. Neither hypophysectomy nor GH administration altered the concentration of serum Na or K.

16. Hyperglycemia and Glycosuria in the Forced-Fed Rat Receiving ACTH and Anterior Pituitary Growth Hormone.

Frank L. Engel and *A. Viau* (by invitation), *W. J. Coggins* (by invitation) and *W. Lynn* (by invitation).

Duke University, Durham, N. C.

Hyperglycemia and glycosuria following treatment of rats with anterior pituitary growth hormone has not been reported heretofore except following partial pancreatectomy or alloxan treatment. In the following study hyperglycemia up to 600 mg. per cent one hour after feeding, and glycosuria up to 7 grams per day, have developed in rats tube-fed a high carbohydrate diet (Ingle) and receiving 5 mg. of growth hormone (Armour) and 3 to 8 mg. of ACTH (Armour) daily intermittently over thirty to one hundred days. At the same level of diet neither ACTH nor GH alone in the above doses induced glycosuria, but blood sugar levels in the GH-treated animals showed transient elevations one hour after feeding. With larger diet volumes GH alone induced hyperglycemia and glycosuria, and with still larger volumes, glycosuria occurred in the

absence of hormone therapy, confirming Ingle. During combined ACTH-GH treatment, with a fixed diet, glycosuria was unchanged by doses of ACTH between 3 and 8 mg. daily, GH being kept constant. However, GH glycosuria was not sustained by 1.5 mg. of ACTH. On a fixed dose of ACTH, glycosuria varied with the dose of GH. It is concluded that GH may be diabetogenic in the intact rat, the adrenal cortex playing a synergistic role.

17. Variations in Cathepsin Activity of Rat Tissues Induced by Hypophysectomy and Replacement Therapy.

James C. Mathies (by invitation), and *Oliver H. Gaebler.*

Edsel B. Ford Institute for Medical Research, Detroit, Michigan.

It has been suggested that cathepsins are involved in protein synthesis in the cell, since they are the only known intracellular proteinases possessing the specificity of action required in elaboration of the complex protein molecule (Fruton, J. S., *Ann. Rev. Biochem.* **16:** 46, 1947). Cathepsin activity in the liver, kidney, and spleen was therefore studied in 5 series of rats. Each series included hypophysectomized untreated and treated animals, and the corresponding normal groups. Following hypophysectomy, reduction in catheptic activity per gram of organ was of a minor order of magnitude in the kidney and spleen, but considerable in the liver. Treatment with large doses of growth hormone slightly increased renal catheptic activity of both normal and hypophysectomized rats, but daily doses of 20 to 80 gamma had no effect. Results with spleen and liver cathepsin also indicated no important relationship to the growth response. Cathepsin activity in the liver of hypophysectomized rats was, however, greatly increased by injections of thyroxin, and the corresponding finding in intact rats, previously reported by others (*Boll. Soc. ital. biol. sper.* **10:** 826, 1935) was confirmed. It would appear that lowering of hepatic cathepsin in hypophysectomized rats is related to deficiency of thyrotropin rather than somatotropin.

18. Effect of Pituitary Hormones on Nerve Function.

Clara Torda, and *Harold G. Wolff* (introduced by Ephraim Shorr).

From the Departments of Medicine, Neurology, and Psychiatry, New York Hospital-Cornell Medical Center, New York, N. Y. and the Kingsbridge Hospital (V.A.), Bronx, N. Y.

After hypophysectomy the function of nerve is impaired, as evidenced by the decrease of action potential during repetitive stimulation and the decrease of ability to synthesize acetylcholine. Muscle function on direct stimulation, however, is not significantly impaired. Administration of ACTH restores the function of nerve to normal in hypophysectomized rats. In the following, an attempt was made to ascertain the effect of other hormones from the pituitary gland on the function of nerve. The latter was tested through measurements of the behavior of action potential and the ability to synthesize acetylcholine by a modified method of Quastel, Tennebaum, and Wheatley. The thyrotropic factor (THS), thyroxin, growth hormone, prolactin, pitocin, and pitressin did not restore to normal the action potential and the ability to synthesize acetylcholine in hypophysectomized rats. These results suggest that of all the hormones used, only ACTH regulates the nerve function in the sense of being instrumental in maintaining optimal nerve function.

19. Some Hormonal Aspects of Water Excretion in Man.

Alexander Leaf and *Audley R. Mamby* (introduced by Fuller Albright).

Massachusetts General Hospital, Boston, Mass.

It has been claimed that anterior pituitary destruction will ameliorate or abolish experimental diabetes insipidus. A patient with diabetes insipidus was observed to lose polyuria and polydipsia spontaneously. Studies revealed insufficiency of anterior and posterior pituitary function. However, the physiologic defect of diabetes insipidus persisted; namely a total inability to produce a hypertonic urine during severe dehydration. The decrease in polyuria reflected merely a decreased total solute load requiring excretion.

Metabolic studies were conducted on a constant diet. Salt and urea administered by increasing the excretory solute load caused return of polyuria. Thyroxine, desoxycorticosterone and cortisone were administered and found to have no effect upon the renal concentrating ability. Hence their alleged diuretic actions are not direct effects but secondary to their action in increasing appetite, metabolism and urinary solute load.

Observations were made on the effects of pitressin administration on electrolyte excretion and upon the interactions of pitressin and cortisone on the ability to excrete ingested water.

20. Evaluation of the Responsiveness to Prolonged ACTH Dosage of a Patient With Panhypopituitarism.

Nelson H. Brown (by invitation) and *R. B. Greenblatt.*

Medical College of Georgia, Augusta, Ga.

A total of 7,495 mg. of ACTH in dosages varying from 25 to 100 mg. per day was administered over a period of one hundred and eighty days to a patient with panhypopituitarism resulting from an expanding chromophobe adenoma. Daily urinary 17-ketosteroid and "cortin" assays were performed during this period, along with urinary creatine and creatinine determinations. Periodic eosinophil and white blood cell counts were made and body weight and well-being were charted.

During the first eighty days variations in circulating eosinophils were in indirect ratio to the dosage of ACTH employed. After this period, however, even increased dosage failed to lower the count in the same degree as was induced earlier in the study. Urinary 17-ketosteroid assays were found to vary directly with ACTH dosage until the hundred and tenth day. Thereafter, the same dose, as well as increased dosage, failed to induce urinary values similar to those previously obtained. Urinary cortins increased directly with the dosage of ACTH but the same and increasing amounts of ACTH did not induce a corresponding output of urinary cortins toward the end of the study. Urinary creatine values gradually decreased over the entire period of study whereas urinary creatinine values, after a preliminary drop, were maintained at as high or a higher level than that at the start of the study. The white blood count increased or fell in direct ratio to ACTH administration during the first one hundred and ten days. Thereafter, the response was not good. The general feeling of well-being was proportional to the dosage up to the hundred and tenth day. After this time, even increased dosage failed to maintain the patient in the same degree of well-being. It appears from this study that some degree of resistance to ACTH responsiveness developed after prolonged therapy. Whether this was due to antibody formation or failure of the adrenal cortex to react to similar or

even moderately increased doses of ACTH or to a temporary exhaustion of the adrenal cortex is yet to be determined.

21. The Use of Cortisone in the Treatment of Young Infants With Congenital Adrenal Hyperplasia and Defective Electrolyte Regulation.

John F. Crigler, Jr. (by invitation), *Lytt Gardner, Claude J. Migeon* (by invitation) and *Lawson Wilkins.*

Johns Hopkins Hospital, Baltimore, Md.

Cortisone therapy has controlled virilization and brought about feminization in female pseudohermaphrodites between the ages of 8 and 18 years. In some infants with congenital adrenal hyperplasia there is deficiency of electrolyte regulation in addition to excessive secretion of androgen. The effects of cortisone on electrolyte regulation are illustrated in the cases of 3 infants with adrenal hyperplasia treated before they were 3 months of age.

22. Accumulation of ACTH in Kidney.

John B. Richards (by invitation) and *George Sayers.*

Department of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah.

A method has been developed for the determination of ACTH activity in tissues. The kidneys of intact uninjected rats contain the equivalent of approximately $\frac{1}{3}$ microgram of ACTH standard (LA-1-A) per 100 grams of rat, as assayed by the adrenal ascorbic acid depletion technic. Rat ACTH was injected intravenously in a dose of 100 microgram-equivalents per 100 grams of rat. ACTH activity in tissue is expressed per 100-gram rat. Five minutes after injection, 20 micrograms had accumulated in the kidneys; 15 micrograms were still present in the kidneys fifteen minutes after injection. The adrenals contained 0.2 and 0.05 micrograms of ACTH activity at five and fifteen minutes, respectively. ACTH activity per unit weight of tissue appeared to be actually less in the adrenal than in the kidney. No ACTH activity could be detected in the liver or in the urine. Preliminary studies suggest that the kidney of hypophysectomized rats accumulates less of the injected ACTH than does the kidney of intact animals. Hog kidney tissue contains appreciable quantities of ACTH activity.

23. Effect of varying Concentrations of ACTH and Growth Hormone Upon Corticosteroid Release From Perfused Adrenals.

I. Macchi (by invitation), *O. Hechter,* and *G. Pincus.*

From the Worcester Foundation for Experimental Biology, Shrewsbury, Mass. and the Department of Physiology, Tufts Medical School, Boston, Mass.

In previous work, we have established that under the influence of *large* doses of ACTH (6 to 70 mg. of Armour LA-1A equivalents per liter), perfused bovine adrenals rapidly synthesize from cholesterol, and possibly other precursors, a variety of corticosteroids (Hechter *et al.: Recent Prog. Hormone Research,* in press; Zaffaroni, Hechter and Pincus: *J. Am. Chem. Soc.,* in press). In the present study, the relationship between ACTH concentration and the total amount of corticosteroid released has been studied with an Armour and with a purified ACTH preparation prepared and furnished us by Dr. Astwood. Studies of the effect of a homogenous growth hormone preparation furnished by Dr. Li, and other hormonal proteins in this corticosteroidogenic system have likewise been undertaken. In all cases a single gland was perfused at a constant flow rate of 1

liter per hour with increasing concentrations of the hormones under investigation, each sample representing different concentrations being collected and analyzed separately. Preliminary results indicate that both samples of ACTH and Li's growth hormone released corticosteroid. Although all preparations showed a sigmoid log dosage-response curve, differences were noted quantitatively with respect to the minimal effective dose (M.E.D.). With the Astwood preparation the M.E.D. is about 0.01 microgram per liter; the Armour preparation exhibits an M.E.D. of about 10 microgram LA-1A equivalents per liter, whereas with Li's growth hormone preparation the M.E.D. is about 100 micrograms per liter. These results indicate that Astwood's preparation is *circa* 50 to 100 times more active than Armour's standard LA-1A based on the direct measurement of corticosteroid release. The surprising finding that the apparently homogenous growth hormone of Li likewise releases corticosteroid, albeit at a concentration approximately 10 times greater than LA-1A, indicates either that the growth hormone moiety itself has the capacity to release adrenal corticosteroids, or that this preparation is contaminated with significant amounts of ACTH activity. Studies to characterize the individual corticosteroids released by varying concentrations of ACTH and other hormonal proteins, are under investigation.

24. The Nature of ACTH Resistance.

Peter H. Forsham, Albert E. Renold (by invitation), and *Thomas F. Frawley* (by invitation).

The Medical Clinic, Peter Bent Brigham Hospital and the Department of Medicine, Harvard Medical School, Boston, Mass.

Only 2 instances of anaphylaxis in over 10,000 injections of Armour ACTH have been observed. Adrenal unresponsiveness as measured by a poor eosinophil fall and 17-ketosteroid rise occurs rarely spontaneously and occasionally after two or more courses. Although a constant intramuscular drip of the same material failed to activate the adrenal cortex, a slow intravenous infusion over an 8-hour period in doses from 10 to 30 mg. was found to yield a normal response. Similarly switching from pork to sheep or beef ACTH intramuscularly led to renewed adrenal activation. Thus, tissue inactivation rather than antihormone formation appears to limit the ACTH response and a local, species-specific sensitivity phenomenon seems to enhance the destruction of ACTH. In the non-resistant subject purified ACTH polypeptides were inactivated more rapidly than commercial ACTH and hyaluronidase enhanced destruction of ACTH locally. Whereas 20 mg. of ACTH intravenously in one minute showed no effect, it doubled 17-ketosteroid excretion when given over forty-eight hours as a slow drip. The effectiveness of ACTH appears determined by the duration of action on the adrenal and the rate of destruction by the tissue.

FRIDAY, JUNE 8, 1951

9:00 A.M.—THYROID GLAND

DRS. RULON W. RAWSON AND ROBERT H. WILLIAMS, PRESIDING

25. Steps in the Secretion of the Thyroid Hormone.

C. P. Leblond.

Department of Anatomy, McGill University, Montreal, Canada.

Previously it has been suggested that "free" thyroxine appears in the colloid of the thyroid follicle as a result of proteolysis of thyroglobulin. The freed thyroxine would

then diffuse into the circulation (Gross and Leblond, 1950, 1951). To study this hypothesis further, three preparations were examined, namely: 1) a butanol extract of the thyroid gland; 2) an alkaline hydrolysate of the butanol extract residue, and 3) a butanol extract of plasma. These fractions were prepared from animals sacrificed at various time intervals after injection of radioiodine, chromatographed on paper, and then radioautographed. The thyroxine spots on the chromatograms of the three extracts were cut out. The radioactivity as well as the total iodine content of each were estimated; and from these the specific activity, that is, the radioactivity per unit weight of thyroxine, was determined. The results showed that, at time intervals up to twenty-four hours after the injection of radioiodine, the specific activity of thyroxine is maximal in the alkaline hydrolysate, intermediate in the butanol extract of the thyroid, and lowest in the butanol extract of the plasma. Since the alkaline hydrolysate represents the thyroglobulin-bound thyroxine, and the butanol extract of the thyroid and plasma represents the "free" thyroxine in these two organs, these findings support the theory that proteolysis of thyroglobulin in the thyroid releases thyroxine, which then finds its way out into the circulation.

26. The Metabolic Fate of Radioactive Iodine in Thyroid Hyperplasia Versus Hyperfunction.

William T. Salter.

Laboratory of Pharmacology, Yale University School of Medicine, New Haven, Conn.

When radioactive iodide is administered to an animal or man, its fate is determined by multiple factors. Chief among these are the renal clearance and the thyroid clearance; but more useful functions for the physiologist and clinician are a) the radioactivity of the exhaled air and b) the specific radioactivity of the circulating (serum) hormone. Even after heavy therapeutic doses, for a few days the isotope behaves like a tracer until damage to the thyroid gland occurs. The exhaled air reflects radioactive iodide (inorganic) in the serum. The blood hormone indicates by its radioactivity the T.M.R. (thyroid metabolic rate), *i.e.*, the amount of *l*-thyroxine produced by the gland per square meter per day. Normally this value is about one quarter of a milligram. In myxedema the value is almost zero and in marked thyroid toxicosis the value is over half a milligram. Single measurements of radioactivity in the neck reflect hyperplasia rather than hyperfunction. Nevertheless, if repeated measurements of radioactivity (over the gland) are made, it is possible to calculate the T.M.R. from the resulting time-curve. These two values for T.M.R. usually agree very well. Moreover they are almost directly proportional to the serum "hormonal" iodine.

27. Metabolism of Radiodiiodotyrosine in the Human.

F. Raymond Keating, Jr.

Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

Radioactive diiodotyrosine labeled with I^{131} was given intravenously in a dose of 1 to 2 mg. to 3 athyreotic patients, brought to a euthyroid level by thyroid therapy. Subsequent observations included total radioactivity, diiodotyrosine, thyroxine and iodide fractions in serum and urine, as well as numerous *in vivo* observations during the ensuing three days. The diiodotyrosine rapidly disappeared from serum, mostly by

excretion into the urine, only 3 per cent of the administered radioactivity appearing in feces. In vivo studies disclosed no selective localization. The initial volume of distribution of diiodotyrosine was 85 cc. per kilogram, but within two hours it diffused into a much larger compartment corresponding to 530 cc. per kilogram. Radiodiiodotyrosine disappeared very rapidly from the blood and was replaced by radioactive iodide. Small quantities of radiodiiodotyrosine were excreted in the urine during the first six hours, but none thereafter. The greatest proportion of the urinary I^{131} consisted of inorganic iodide. The renal clearance of radiodiiodotyrosine averaged 5.2 cc. per minute, which is one fifth the renal clearance of the inorganic iodide liberated from it. Approximately 200 cc. of serum was cleared per minute of its diiodotyrosine content by diiodination which, therefore, appears the principal route for disposal of the substance.

28. The Nature of the Blood Iodine Following Large Doses of I^{131}.

J. Robbins, D. V. Becker, and *J. E. Rall* (introduced by Rulon W. Rawson).

Memorial Center for Cancer and Allied Diseases, New York, N. Y.

Previous studies by others have shown that, following a small dose of radioiodine in a normal or hyperthyroid individual, about 95 per cent of the serum I^{131} is protein-precipitable and about 80 per cent is butanol-soluble. There is evidence to indicate that this serum iodine is in the form of thyroxine, loosely bound to protein. It has also been observed that a large dose of radioiodine which brings about destruction of thyroid tissue, may be followed by a rise in the chemical protein-bound iodine, and that this is sometimes accompanied by hyperthyroidism.

In this study, the nature of the blood iodine following large doses of radioiodine was investigated. It was found that whereas about 95 per cent of the serum iodine was protein-precipitable, well under 50 per cent was butanol-soluble. It was shown by chromatographic techniques that the butanol-soluble fraction was probably identical with thyroxine. Furthermore, a thyroxine-like compound was identified in the butanol-insoluble fraction after hydrolysis with strong alkali. These findings indicate that the increase in circulating iodine following destruction of thyroid tissue with radioiodine is in large part due to the discharge into the blood of a complex molecule from which thyroxine can be released by hydrolysis.

29. Relationship of Gonadal Imbalance to Thyroid Disease—A Statistical Study.

Francis P. Ferrer and *Thomas H. McGavack.*

From the Department of Medicine, New York Medical College, New York, N. Y.

The purpose of the study is to determine the relationship of gonadal dysfunction and pathology to the incidence and exacerbation of thyroid disease.

In approximately 500 cases of thyroid disease, there was an associated incidence of fibromyoma uteri in 20 per cent and some type of pelvic pathology in 45 per cent. In the thyroid disturbances of puberty, some gonadal imbalance was demonstrable in 80 per cent. A high percentage of unnecessary surgery was performed in these subjects.

There is a tendency for subjects with borderline thyroid disease to show cardiac manifestations early, with a frank flare-up in the thyroid as a terminal finding.

The toxic nodular goiters (15 per cent) show a less frequent association than the nontoxic adenoma (30 per cent), with gonadal pathology. Malignancy of the thyroid is rarely associated with gonadal pathology (1 to 2 per cent).

The frequency of gonadal dysfunction, followed or preceded by thyroid disease is not pure coincidence but rather follows a definite trend. The entire endocrine pattern of every patient with thyroid disease should be fully considered in our approach to therapy if he or she is to avoid useless surgery, unnecessary complications and prolonged periods of chronic ill-health.

30. Ovarian Function in Thyrotoxicosis and Hypothyroidism.

Richard Goldsmith (by invitation), *Sommers Sturgis* (by invitation), *John B. Stanbury* (by invitation) and *Jacob Lerman.*

Thyroid Clinic, Massachusetts General Hospital, Boston, Mass.

Ovarian function was studied in 27 premenopausal females who had well documented syndromes of thyroid dysfunction. The patients were divided into three groups: (a) 7 patients with myxedema of from two months' to nine years' duration; (b) 5 patients with definite hypothyroidism without clinical myxedema; (c) 15 patients with thyrotoxicosis, 2 of whom developed post-therapy hypothyroidism. Three patients with myxedema had clinical metropathia hemorrhagica, 1 had amenorrhea and 3 had menorrhagia. Five of these patients were found to be menstruating from proliferative endometria, 1 from a very early secretory endometrium and 1, myxedematous for only two months, from a late secretory endometrium. Three of these patients' urines were examined for pregnandiol: none was present. The clinical and objective abnormalities noted in the myxedema group have uniformly reverted to normal during adequate thyroid therapy in the 4 patients treated to date.

Ten of the subjects with hyperthyroidism had oligomenorrhea, 3 had amenorrhea, and 2 had normal periods. Each patient with amenorrhea had a proliferative endometrium whenever examined and failed to excrete urinary pregnandiol; the patients with oligomenorrhea apparently ovulated normally. In this group, also, the abnormalities disappeared when the patients became euthyroid.

31. Effect of Massive Cortisone Therapy on Thyroid Function.

D. S. Frederickson (introduced by Peter H. Forsham).

The Medical Clinic, Peter Bent Brigham Hospital and the Department of Medicine, Harvard Medical School, Boston, Mass.

Of 180 patients with Addison's disease, 4.4 per cent showed hyperthyroidism. In contrast, marked suppression of thyroid function in euthyroid individuals was demonstrated during massive cortisone therapy. Twelve patients received 500 mg. of cortisone daily for several weeks. Changes in thyroid function were measured by protein-bound iodine (PBI), tracer doses of radioactive I^{131} followed by in vivo counting over the gland for at least twenty-four hours, and urinary excretion studies.

A male rheumatoid given 50 microcuries of I^{131} intravenously before treatment accumulated 21 per cent of the tracer in twenty-four hours. This fell to 3 per cent on the fourth day of intramuscular cortisone; and the PBI fell from 5.9 to 2.2 gamma per cent. On the sixth day of treatment 50 mg. of TSH (Armour H-2103) increased the 24-hour accumulation to 10 per cent. PBI levels remained below 3 gamma per cent for over eight weeks after cessation of therapy. The suggestive finding of a ten times greater incidence of hyperthyroidism in Addisonians as compared to normal subjects and the complete suppression of thyroid activity by massive doses of cortisone further emphasize the functional adrenal-thyroid interrelationship.

32. Modification of TSH Action by ACTH and Cortisone in Hypophysectomized Rats.
Dixon M. Woodbury (by invitation), *B. N. Ghosh* and *George Sayers.*

Department of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah.

Beginning the third day after hypophysectomy, male rats, weighing 100 to 150 grams, were injected subcutaneously every eight hours for a total of 8 doses with TSH, ACTH and cortisone, alone or in various combinations. I^{131} was injected intraperitoneally at the time of the seventh injection and the rats were sacrificed twenty-four hours later. In a first experiment, percentages (with S.E.) of the administered dose of I^{131} taken up by the thyroid were as follows: controls, 21.09 ± 4.31; ACTH alone (5 mg.), 23.64 ± 3.32; TSH alone (0.5 mg.), 70.87 ± 2.10; and ACTH (5 mg.) plus TSH (0.5 mg.), 43.62 ± 2.10. In a second experiment, corresponding values were: controls, 14.08 ± 0.84; cortisone alone (8 mg.), 12.04 ± 1.01; TSH alone (0.5 mg.), 60.37 ± 4.74; and TSH (0.5 mg.) plus cortisone (8 mg.), 30.91 ± 2.77. An increased rate of excretion of I^{131} was associated with the reduced uptake of I^{131} by the thyroid after ACTH or cortisone. ACTH in combination with TSH markedly inhibited the increase in PBI^{131} which normally occurs when TSH is given alone. The effect of cortisone on PBI^{131} was equivocal. Possible interpretations of these experiments are discussed.

33. Blood Thyroid and Thyrotropic Hormone Levels in Endocrinopathy.
S. A. D'Angelo, K. E. Paschkis, A. Cantarow and *A. S. Gordon.*

Jefferson Medical College, Philadelphia, Pa.

The status of anterior pituitary-thyroid gland interplay in normal subjects and in patients with thyroid or pituitary disorders has been studied by determining the levels of thyroid and TSH activities in untreated sera (stasis tadpole technic, D'Angelo and Gordon, *Endocrinology* **46:** 39, 1950) and correlating these with the clinical state. In normal serum, TSH activity was of a low order of magnitude (range, 0 to 0.001 JSU/ml. serum) with thyroid hormone activity relatively high. In acromegaly (3 cases), blood titers of TSH were increased approximately 8-fold with thyroid hormone levels remaining near normal limits. Neither thyroid hormone nor TSH was demonstrable in the blood of patients with Simmonds' disease. Blood thyroid hormone activity in hypothyroidism (10 cases) was consistently low but TSH titers varied widely. In hyperthyroidism (10 cases), excessively high thyroid hormone levels in blood usually co-existed with normal TSH content. The thyroid-TSH balance in ophthalmopathy (8 cases) was essentially normal, although elevated TSH levels did occur (2 cases). Clinical improvement in exophthalmic and hypothyroid individuals (with desiccated thyroid) could be effected in the presence of high blood titers of TSH. The results indicate that the thyroid-TSH balance in the circulating blood is frequently altered in individuals with thyroid or pituitary malfunction, and that amelioration of clinical symptoms can occur without restoration of the hormonal balance to normal.

34. A Comparison of the Effect of Thyrotropin on Iodine Metabolism in Euthyroid and Hyperthyroid Humans.
D. V. Becker, J. E. Rall and *W. C. Peacock* (introduced by Leonard P. Eliel).

Memorial Center for Cancer and Allied Diseases, New York, N. Y.

Three euthyroid and 4 hyperthyroid patients were given tracer doses of I^{131} and measurements were made of the total serum I^{131}, serum precipitable I^{131}, radioactivity

over the thyroid gland, and urinary radioiodine daily and at frequent intervals after the injection of 40 mg. of thyrotropin. The thyrotropin was given intramuscularly when a plateau in the serum precipitable I^{131} had been reached (within six days after the tracer had been administered). Potassium thiocyanate was administered beginning twenty-four hours after the tracer dose of I^{131}, to prevent reincorporation of catabolized thyroid hormone iodine in the thyroid gland. A markedly increased urinary excretion of I^{131} was noted in the twenty-four hours following the administration of thyrotropin. Similar, though less marked elevations were seen in the serum precipitable I^{131} and the chemical protein-bound iodine. In vivo thyroid measurements showed inconsistent change. In 1 patient with acromegaly and recurrent hyperthyroidism the injection of thyrotropin was followed by no change in any of the indices of iodine metabolism measured. In all other patients studied, administration of thyrotropin resulted in the release of significant quantities of thyroid hormone. There were no consistent quantitative differences between euthyroid and hyperthyroid patients.

35. Inhibition of Thyroid Function by Struma Ovarii: Effect of TSH on Normal and Inhibited Thyroid.

Martin Perlmutter, Shirley Weisenfeld (by invitation), *Eleanor A. Zucker* (by invitation) and *Stanley L. Slater* (by invitation).

Medical Services of the Maimonides Hospital and The Department of Medicine, State University Medical Center at New York, Brooklyn, N. Y.

Serial studies of thyroid function were made after the removal of a struma ovarii. Serum PBI fell from high normal to subnormal levels and gradually rose to normal. The cervical thyroid accumulated no I^{131} for three weeks. During the sixth week, normal I^{131} uptake was noted. Chemical analysis of the struma revealed significant concentration of PBI. Histologic studies revealed thyroid tissue. These data suggest that a functioning struma ovarii depressed the cervical thyroid, that removal of the ovary resulted in a rapid decline of serum PBI, and that the low serum PBI released the cervical thyroid from its inhibited state. A similar inhibition of thyroid function has been reported following the ingestion of thyroxin.

To elucidate the mechanism of this type of thyroid inhibition, the response of 4 normal subjects to the injection of TSH was studied both before thyroid ingestion and when the thyroids were inhibited by ingested thyroxin. The effects of TSH, as measured by increase in PBI and uptake of I^{131}, were the same in both periods. These results indicate that direct inhibition of the thyroid's ability to respond to TSH is not the cause of depression of normal human thyroid function following thyroxin ingestion.

36. The Effect of Hypophysectomy on Intrathyroidal and Extrathyroidal Metabolism of I^{131}.

A. Albert.

Mayo Foundation and Mayo Clinic, Rochester, Minnesota.

The effect of hypophysectomy on iodine metabolism in male rats maintained on an iodine-deficient diet has been studied with the aid of I^{131}. Following hypophysectomy, I^{131} uptake rapidly declined so that by the fifth day only 10 per cent of that normally collected was accumulated by the thyroid. This decrease in the collection occurred in face of a lowered renal excretion of iodide and a higher blood concentration in hypophysectomized than in normal animals. The rate of protein-binding of I^{131} in hypophysecto-

mized rats was slower than normal, but eventually 95 per cent of the I^{131} collected became protein-bound. The proportions of acid-soluble and acid-insoluble I^{131} after hydrolysis of the thyroids were different in hypophysectomized animals, to the extent that there was proportionally less thyroxine being formed from its precursor, diiodotyrosine. The rate of discharge of thyroid hormone, as determined directly by an in vivo counter in rats hypophysectomized for five days, was a tenth that of normal rats. These observations indicate that hypophysectomy induced no qualitative changes in intrathyroidal or extrathyroidal metabolism of iodine. Hypophysectomized rats collect iodine, transform it to hormone, and discharge hormone into the blood at rates which, when measurable, approximate a tenth that of nonhypophysectomized animals.

37. Pathologic Characteristics of the Thyroid Gland Prepared for Surgery With Iodo-Thiouracil and Other Antithyroid Drugs.

Boris Catz (by invitation), *Weldon K. Bullock* (by invitation), and *Paul Starr.*

Departments of Medicine and Pathology, University of Southern California and The Thyroid Clinic, Los Angeles County Hospital, Los Angeles, California.

The routine pathologic features of the thyroid gland, analyzed as to size of follicle, colloid quality, lymphoid tissue, hyperplasia, interstitial stroma, and vascularity, were studied in glands removed by surgery from patients prepared, in 18 cases with sodium 5-iodo-2-thiouracil, in 24 cases with Lugol's, and in 30 cases with various antithyroid drugs plus iodine. Additional measurements were made of the mean acinar cell height by microhistometry. Similar observations were also made in 20 normal thyroids as controls. The impression is gained thus far that the glands prepared with sodium 5-iodo-2-thiouracil were less vascular, have less lymphoid tissue, and have a lower mean acinar cell height than those treated with Lugol's alone. The usual microscopic means of reporting the degree of hyperplasia as mild, moderate, or marked has been found not to be as accurate as the microhistometric determination of the mean acinar cell height. These studies are part of a project to demonstrate unequivocally whether or not the action of sodium 5-iodo-2-thiouracil is actually different from other antithyroid drugs combined with iodine.

38. Effects of Sodium Iodide, Propyl Thiouracil, and Methyl Mercaptoimidazol During Radioiodine Therapy of Hyperthyroidism.

Howard Hamilton (by invitation) and *Sidney C. Werner.*

Department of Medicine, Columbia University College of Physicians and Surgeons, and the Presbyterian Hospital, New York, N. Y.

Approximately four to eight weeks elapse between radioiodine administration in hyperthyroidism, and clinical effect. The use of sodium iodide, propyl thiouracil, and methyl mercaptoimidazol in this interim has been investigated as a means of hastening control over toxicity. Seventy-nine hyperthyroid patients have been treated in four therapeutic groups: 1. Radioiodine alone. 2. Isotope and sodium iodide. 3. Isotope and propyl thiouracil. 4. Isotope and methyl mercaptoimidazol. Drug therapy was started in general one week after I^{131} administration and was continued for two to three weeks. The effective half-life of the isotope in the thyroid, and the level of serum precipitable iodine are reported. The effective half-life is substantially unaltered from control values in all groups. Serum precipitable iodine levels for two to three weeks after radioiodine administration are reduced only in the methyl mercaptoimidazol group. Serum values are

lowered in all groups at six weeks to four months, but this decline is least after sodium iodide. Less success in achieving ultimate remission is noted with sodium iodide and propyl thiouracil, although temporary remission was best induced by sodium iodide and methyl mercaptoimidazol. The therapeutic significance of these findings is discussed.

2:00 P.M.—GONADS AND REPRODUCTION

DRS. EARLE T. ENGLE AND E. C. HAMBLEN, PRESIDING

39. Demonstration of a Nonestrogenic, Uterine Stimulating and Estrogen Augmenting Substance in Urine from Pregnant Mares.

Herman Cohen and *Robert W. Bates.*

Department of Biology, New York University Washington Square College, New York, N. Y., and Squibb Institute for Medical Research, New Brunswick, N. J.

Fractionation of the nonketonic phenolic fraction of pregnant mares' urine into 0.1 N NaOH, 1 N NaOH, and neutral fractions resulted in an apparent loss of 50 per cent of the estrogenic activity when each fraction was assayed by the vaginal cornification method. However, recombination of the fractions restored the original activity. Further combination studies indicated that the neutral fraction contained materials which have no estrogenic activity as measured by the vaginal cornification method, but which double the estrogenic activity of natural estrogens as well as of stilbestrol. As little as 1.5 micrograms, when given by mouth or by injection, doubled the potency of estrogen injected separately. When 56 micrograms of the augmenting material alone were injected daily for four days into adult ovariectomized rats, only negative estrus smears were found, demonstrating the absence of estrogenic activity.

When 28 micrograms of this augmenting substance were injected subcutaneously daily for three days into intact, castrate, castrate-adrenalectomized, and hypophysectomized immature female rats, the uterine weight was doubled. This increase was equivalent to that obtained with 4 micrograms of estrone injected daily for three days. There was no augmentation of uterine weight when estrone and the augmentor were combined.

Isolation studies are in progress.

40. Identification of Steroids in the Human Testis.

Joseph W. Goldzieher and *Irene S. Roberts* (by invitation).

From the Research Department, St. Clare's Hospital, New York, N. Y.

Attempts to identify and isolate testicular hormones have been limited in the past to the boar, bull and stallion. There has been no reported work on human material. Recent discussions of estrogen as a possible "second testicular hormone" in man have been based largely on indirect evidence, observations on animals, or studies of abnormal tissues. Investigation of normal human testicular parenchyma, therefore, seems essential. Accordingly, 688 testes containing 8.75 Kg. of such tissue were obtained from healthy young adults dying of acute trauma. This material was subjected to an extensive isolation procedure intended to separate out phenolic and neutral steroids. The latter fraction has been separated into ketonic, nonketonic; alpha, beta fractions, and identification of these fractions is in progress.

The phenolic fraction was separated into "estriol," "estradiol" and "estrone" fractions. Bioassay showed activity only in the "estradiol" fraction and paper chromatography yielded material with the characteristic Rf of estradiol. It is estimated that there

is a minimum of 5.7 micrograms of estradiol per kilo in human testicular parenchyma, or approximately 0.086 microgram per testis.

41. Studies on the Metabolism of Progesterone.

John G. Wiswell (introduced by Leo T. Samuels).

Department of Biological Chemistry, College of Medicine, University of Utah, Salt Lake City, Utah.

Following the injection of progesterone into man, pregnanediol is excreted in the urine However this accounts for less than one third of the progesterone administered, and the fate of the remainder is unknown. It has been postulated that the liver is the site of inactivation of progesterone, since the compound is much less active when placed so as to pass through the liver than when given subcutaneously. We have incubated 2 micromoles of progesterone with 0.3 to 1.0 gram of fresh liver tissue from the rat, dog, and human, at 37.5° C. for one hour in buffered Locke's solution. The amount of steroid was estimated by the absorption maximum at 240 mμ. The rate of destruction varied among species. Disappearance of the conjugated double bond system was increased by citrate, but not by diphosphopyridine nucleotide. Neither formation of 17-ketosteroids nor of free pregnanediol could be demonstrated. Radioactive progesterone is being employed in an attempt to discover what compounds progesterone yields when metabolized by tissues.

42. Uptake of P^{32} in Seminal Vesicles of Castrate Rats After Treatment With Testosterone Propionate.

Walter Fleischmann and *Susan Kann Fleischmann* (by invitation).

Radioisotope Unit, Veterans Administration Hospital, Fort Howard, Maryland.

Male Wistar rats were castrated before puberty and used from seven to thirty-five days afterwards. One group of these rats was given 2.5 mg. of testosterone propionate (TP) in sesame oil subcutaneously (TP rats). Seventy-two hours after injection these rats received about 50 microcuries of P^{32} in water subcutaneously. Another group received P^{32} only (control rats). All animals were killed five hours after administration of P^{32}, the seminal vesicles removed, digested with KOH, dried and their activity determined. Activities are expressed as counts/minute/mg. of fresh tissue. The activity of the seminal vesicles of the TP rats was 74.6 ± 2.5 and that of the control rats 20.4 ± 3.9. Seminal vesicles were fractionated according to the method of Schmidt and Thannhauser. The fraction containing desoxyribonucleic acid showed activity of 4.80 ± 0.68 in the TP rats and 0.28 ± 0.19 in the control rats. The fraction containing ribosenucleic acid showed activity of 16.2 ± 2.4 in the TP rats and 3.4 ± 0.9 in the control rats. The relationship of increased uptake of P^{32} to the growth of the seminal vesicles in the rats treated with TP is discussed.

43. Effect of Purified Pituitary Gonadotropins in the Hypophysectomized, Immature Male Rat.

C. A. Schaffenburg (by invitation) and *E. P. McCullagh.*

Cleveland Clinic Foundation and the Frank E. Bunts Educational Institute, Cleveland, Ohio.

The specific androgen-responsiveness of the immature and castrate rat's prostatic phosphatases has been previously established, in our laboratory, to be a more sensitive index of androgenic activity than the weight of the sex accessories alone. The purpose

of the present study was to determine whether this response is also specific for ICSH and if so, whether it could form the basis of a test for the measurement of this hormone.

After studying the effects of purified pituitary preparations (FSH33HM3, LHL47HM3, Dr. R. K. Meyer; Prolactin #71713, Dr. R. W. Bates) in the hypophysectomized, immature male rat, the following conclusions were arrived at: a) the response is specific for ICSH; b) FSH or prolactin alone are inactive in this respect; c) addition of FSH to ICSH slightly synergizes the effect of the latter; d) the addition of prolactin to the former combination slightly inhibits the response. The histologic findings showed a good correlation with the above data. Determination of total cholesterol in the testis showed no change in the absolute amounts of this substance, although FSH caused a slight decrease in concentration.

44. The Acid Phosphatase Concentration of the Prostatic Exprimate During Normal Puberty.

John Esben Kirk (by invitation), *Albert Eisenstein* (by invitation) and *Cyril M. MacBryde.*

Department of Medicine, Washington University School of Medicine, St. Louis, Missouri.

A simple rapid method of measuring androgenic function is greatly needed. Acid phosphatase with a pH optimum of 4.9 is found in high concentration in the prostatic secretion of normal men, the mean for age 20 to 40 being 14,400 units per ml. with a gradual decline thereafter. The high concentration of enzyme in secretions from the prostate seems to constitute a true secondary sex characteristic. Only a few determinations of the acid phosphatase level in exprimates from boys have been reported. Our study was undertaken to determine the rise in acid phosphatase concentration of the prostatic secretion during normal puberty, and includes observations on 71 normal boys aged 11 to 16½ years. Phosphatase assays were done by From Hansen's method, sodium phenol phosphate being used as substrate for the enzyme. Results showed the following mean phosphatase values expressed in units per ml. exprimate: 11–12 years, 50; 12–13 years, 200; 13–14 years, 2,880; 14–15 years, 7,020; 15–16 years, 7,380; and 16–16½ years, 8,700. Correlations have been made between observed phosphatase values and clinical signs of sexual development.

45. Some Metabolic Studies With 17-Methylandrostenediol.

Jonas Weissberg, Jacqueline E. Chevalley, Sidney Stern and *Thomas H. McGavack.*

From the New York Medical College, Metropolitan Hospital Research Unit, New York, N. Y.

External balances for nitrogen, sodium, potassium and chloride have been determined in 11 subjects receiving doses of 17-methylandrostenediol in amounts of 500, 250 and 100 mg. daily, for periods varying from seven to eight days. "Fore" periods for control purposes of four days' duration have been employed, and the study has been continued for periods of time varying from four to fifteen days after cessation of steroid administration. Simultaneously, the urinary excretions of creatinine, uric acid and 17-ketosteroids have been quantitatively determined. Fasting blood determinations have been carried out for cholesterol, nonprotein nitrogen, urea nitrogen, creatinine, uric acid, albumin, globulin, sugar, sodium, potassium, chloride, calcium and phosphorus at periodic intervals throughout the study. The clinical condition of the patients has been observed and

weights have been recorded daily or oftener. 17-Methylandrostenediol, at every dosage level tried, caused a retention of nitrogen, sodium and chloride.

46. Variations in Blood Lipids and Lipoproteins Throughout the Menstrual Cycle.

Hyman Engelberg (introduced by Samuel Glass).

Cedars of Lebanon Hospital, Los Angeles, California.

The blood cholesterol, cholesterol esters, phospholipid, neutral fat and total lipids were determined in 10 women who had regular menstrual cycles. Determinations were made at weekly intervals throughout the cycle. All samples of blood were also analyzed by the ultracentrifuge method at the University of California at Berkeley for alpha and beta lipoproteins according to the method described by Dr. John Gofman and associates. Previous similar studies, necessarily not as complete, are discussed, as are hormone relationships to the variations in lipid concentration and to the problem of atherosclerosis.

The cyclical changes in beta lipoproteins analyzed by the ultracentrifuge method show considerably more variation in the cycle than do the results of the ordinary lipid analyses.

47. Klinefelter Syndrome: A Study in Five Cases. Histophysiologic Basis for a Pathogenic Interpretation.

F. A. de la Balze, F. C. Arrillaga (by invitation), *J. Irazu* (by invitation) and *R. E. Mancini* (by invitation).

Facultad de Ciencias Médicas, Buenos Aires, Republica Argentina.

Five cases of Klinefelter syndrome were studied, showing the clinical and hormonal characteristics described by other authors. Histochemical techniques were applied to the testicular material in order to give a histophysiologic basis to the clinical and hormonal findings. For purposes of comparison, these methods were also applied to 16 normal testes. As a consequence, the following etiologic and pathogenic interpretation is proposed: at puberty, due to genetic reasons, a disturbance in the normal differentiation of the Leydig cells occurs; few attain the adult secretion stage. This deficiency in androgenic action (specially local) causes a lack of equilibrium between the intertubular connective tissue and the tube wall. Fibroblasts proliferate and produce abundant reticulin fibers and later on, collagen fibrils. The progressive lesion of the wall leads to atrophy of the spermatic and the Sertoli cells and increased FSH excretion. Alternate hypothesis: For genetic reasons, the "whole" testicular connective tissue is deficient, producing the previously described lesions.

48. The Treatment of Uterine Bleeding by Intravenous Administration of Estrogen.

Robert B. Greenblatt and *W. E. Barfield* (by invitation).

Medical College of Georgia, Augusta, Ga.

Sixty episodes of acute uterine bleeding were treated by the intravenous administration of an aqueous solution containing Premarin (R) (Conjugated Natural Estrogens—equine). The usual dosage employed was 5 cc. (containing 20 mg. of estrogenic substances expressed as sodium estrone sulfate) every six to twelve hours for a total of 3 or 4 doses.

Persistent efforts to determine the cause of menorrhagia included complete medical histories and physical examinations, complete blood counts, capillary fragility tests, and in many cases, endometrial biopsy by aspiration curettage, pregnancy tests and repeated daily pelvic examinations with particular attention to evaluation of response to therapy.

The response to intravenous estrogen therapy was observed in menorrhagia associated with various conditions, including functional uterine bleeding associated with cystic glandular hyperplasia of the endometrium, acylic and imperfect progestational endometrium, uterine fibromyomas, estrogen-withdrawal bleeding, progesterone-withdrawal bleeding (excessive, in presence of fibromyomas), endometriosis, endometrial polyp, Von Recklinghausen's disease, menopausal menorrhagia, pelvic inflammatory disease; also from organic causes, including menorrhagia associated with post-abortal subinvolution of the uterus, ectopic pregnancy, and adenocarcinoma of the body of the uterus.

Satisfactory arrest of bleeding was noted in three to twelve hours after initial intravenous injection of 20 mg. of Premarin (R) in 93.9 per cent of cases (56 out of 60 instances).

After initial arrest of bleeding by administration of estrogens intravenously, a regimen of oral estrogen therapy in diminishing doses followed by a course of cyclic progestin therapy was satisfactory in re-establishing the menstrual cycle.

No untoward effects were encountered from intravenous administration of conjugated estrogens.

From the results of this study it may be concluded that estrogens prepared for intravenous administration may be successfully employed in the treatment of functional uterine bleeding. This form of therapy is particularly valuable for certain cases in which withdrawal bleeding is undesirable.

49. The Effects of Chorionic Gonadotropin and Luteotropin on the Maintenance of Corpus Luteum Function.

Paul H. Fried and *A. E. Rakoff.*

Division of Endocrine and Cancer Research, and the Departments of Obstetrics and Gynecology, Jefferson Medical College and Hospital, Philadelphia, Pa.

The effects of a combination of chorionic gonadotropin and lactogenic hormone on corpus luteum function were evaluated in 8 normally menstruating sterility patients on the basis of prolongation of corpus luteum activity and correction of irregularities of the endometrium. In all cases the endometria were of the mixed or secretory type, with glycopenia. In some cases the postovulatory phases of the menstrual cycles were shortened. The luteal effects were evaluated by studies of the basal temperature records, the endometrial biopsy tissues, and the urine pregnandiol levels in some patients. The minimal effective luteotropic combination of hormones, as demonstrated in 4 cases, consisted of 2,000 I.U. of chorionic gonadotropin and 200 units of lactogenic hormone. This treatment evoked a persistence of corpus luteum function for as much as thirteen days beyond the expected date of menses, and a change to a normal type of endometrium. Studies revealed that the effects were mediated by a functional corpus luteum. In the discussion, consideration is given to luteotropin as a gonadotropin in the hormonal interrelationship of the pituitary and ovary, to the use of the combined therapy in selected cases of sterility and habitual abortion, and to menorrhagia due to persistent corpus luteum function.

50. Therapy of Eunuchoidism With Chorionic Gonadotropin: Aqueous Solution Compared With an Oil and Beeswax Suspension.

Frederic C. Bartter, Ronald Sniffen (by invitation), *Fred Simmons* (by invitation) and *Fuller Albright.*

Harvard Medical School, Boston, Mass.

The effects of chorionic gonadotropin (APL, Ayerst) were studied in 10 male eunu-

choid subjects, comparison being made between an aqueous solution and an oil-and beeswax suspension. All subjects initially showed a low or absent urinary follicle-stimulating-hormone titer, a span greater than the height, underdevelopment or absence of secondary sex characteristics, and testicular biopsy findings similar to those of the prepubertal state. Urinary 17-ketosteroids, serial testicular biopsy specimens, and, where possible, spermatozoa counts were used to measure therapeutic effects.

In each case APL produced a rise in 17-ketosteroids and maturation of the testicular structure. The oily preparation given at five-day intervals appeared to be as effective, unit for unit, as the aqueous preparation given daily. There was reversion towards the initial state in all patients in whom therapy was stopped, with 1 exception. In 2 patients treatment was continued for thirteen and nineteen months with production of spermatozoa and a normal testicular pattern. The wife of one became pregnant. When these patients had reverted to the initial state on cessation of therapy, methyltestosterone was ineffective in restoring spermatogenesis. In 1 case treatment with methyltestosterone was continued for eight months after cessation of APL, with development of normal spermatozoa counts and testicular structure. In this patient the changes persisted upon cessation of all therapy.

51. Increased Estrogen Excretion Due to Stimulation of Leydig Cells in Adult Men Receiving Chorionic Gonadotropin.

William O. Maddock and *Warren O. Nelson.*

Wayne University College of Medicine, Detroit, Mich., and the State University of Iowa, College of Medicine, Iowa City, Iowa.

Although it has been presumed that the testis elaborates estrogen, whether this estrogen arises from Sertoli, Leydig, or germinal cells remains a moot point. A marked increase in urinary estrogen excretion was encountered in adult men receiving chorionic gonadotropin. Therefore, changes in testicular morphology were correlated with urinary estrogen levels in 5 men, aged 33 to 67, to whom chorionic gonadotropin, 5,000 international units three times weekly, was administered for seven to nine weeks. Estrogen levels which were initially low or normal, increased four to sixteen times. This increase occurred within one week, was maintained throughout therapy, and disappeared one week after stopping therapy. Gynecomastia occurred in 2 patients. Urinary 17-ketosteroids increased about twofold, but decreased towards pretreatment levels during the second month of therapy. Neither estrogens nor 17-ketosteroids increased when chorionic gonadotropin was administered to a recently traumatically castrated 45-year-old man. Repeat testicular biopsy specimens obtained in 3 patients at the end of therapy each revealed a marked increase in numbers of Leydig cells, no significant changes in Sertoli cells, a decrease in spermatogenesis, and an increase in fibrosis of the tubules. It is concluded that the Leydig cells are the source of estrogen elaborated by the human testis.

SATURDAY, JUNE 9, 1951

9:00 A.M.—ADRENAL GLANDS

DRS. GREGORY PINCUS AND GEORGE W. THORN, PRESIDING

52. Enzymic Hydrolysis of Urinary Corticoids.

Eleanor H. Venning.

McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

The enzymic hydrolysis of urine by means of a purified preparation of spleen glucu-

ronidase leads to the release of large amounts of corticoids that can be measured by chemical and biologic procedures. In the present study the urinary corticoids have been determined in normal individuals and in patients receiving ACTH and cortisone and the results have been compared with those obtained by previous methods. In the normal individual the total amount of neutral urinary formaldehydogenic corticoids, after enzymic hydrolysis, ranges from 8.5 mg. to 21.2 mg. per twenty-four hours, as compared with 0.5 mg. to 1.8 mg. per twenty-four hours obtained by extracting the urine at pH 1. The excretion of corticoids in a patient receiving 100 mg. of cortisone daily was 24.4 mg. per twenty-four hours using enzymic hydrolysis, whereas only 3.8 mg. per twenty-four hours was obtained on extraction of the urine at pH 1.

Studies are presented showing the excretion of the conjugated corticoids following administration of ACTH, and methods are discussed.

53. A Chemical Method for the Determination of Adrenal Steroids in Peripheral Blood of Human Patients.

Don H. Nelson (by invitation), *Donald G. Willardson* (by invitation), *Frank H. Tyler* and *Leo T. Samuels.*

University of Utah College of Medicine, Salt Lake City, Utah.

A micro modification of the color reaction of Porter and Silber which is specific for 17,21-dihydroxy-20-ketones has been adapted to a method of extraction and isolation of adrenal steroids from blood. This method has made possible the measurement of these adrenal steroids quantitatively in 20 to 30 ml. of peripheral blood. The normal levels for these steroids have been found to be 4 to 6 micrograms per 100 ml. in peripheral venous blood and 9 to 11 micrograms per 100 ml. in arterial blood of human beings.

Preliminary investigations of the effects of stress or single intravenous doses of ACTH upon the levels of these substances demonstrate an immediate increase in both levels. Adrenal steroids in peripheral venous blood have been shown to increase markedly following daily ACTH administration to rheumatic patients and others receiving ACTH therapy.

54. Adrenal Cortical Function as Revealed by Histochemical Tetrazolium Studies.

B. W. Zweifach, M. M. Black and *E. Shorr.*

Department of Medicine, Cornell University Medical College, and Department of Pathology, New York Medical College, New York, N. Y.

The desirability of a histochemical procedure which would provide an index of the activity metabolism of the adrenal cortex led to the exploration of the potential usefulness of 2,3,5-triphenyl tetrazolium chloride (TTC). This reagent is a colorless, water-soluble compound which, upon reduction by dehydrogenases, is transformed into a red insoluble formazan by the action of living cells and deposited intracellularly in characteristic patterns. Surviving adrenal slices were incubated in vitro with TTC for one hour at 37° C., and fixed with 10 per cent neutral formalin; frozen sections were then prepared for microscopic study. Under normal conditions, the three zones of the adrenal cortex become uniformly stained with reduced TTC in a characteristic manner. Following hypophysectomy, there is a progressive impairment of the capacity to reduce TTC in the fasciculata and reticularis, whereas the glomerulosa continued to reduce TTC actively. Acute depletion of the adrenals by cold, hemorrhage and ACTH, results in marked im-

pairment or complete suppression of TTC deposition in the fasciculata and reticularis. The relation of specific hormones to their zones of origin was studied in rats and dogs injected with DCA, cortisone and compounds S and F. The implication of this type of approach to the problem of adrenal cytophysiology is discussed.

55. Observations on the Conversion of Cortisone to Other Corticosteroids.

Robert B. Burton, Christine Waterhouse (by invitation) and *E. Henry Keutmann.*

Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

In a patient with leukemia whose urine was analyzed by paper chromatography, the administration of cortisone acetate resulted in a three to fourfold rise in excretion of cortisone as well as compound F and a more polar alpha-ketolic component. This suggested the conversion of cortisone to these other compounds. Cortisone acetate was administered to 2 patients known for years to have classic evidence of advanced Addison's disease. For over a month before, and during the experiments no therapy was used except maintenance doses of DOCA. Metabolic balance studies indicated no response to 100 mg. of ACTH daily given before cortisone. During control periods the first patient excreted free corticosteroids in trace amounts. On 100 to 200 mg. of cortisone daily for twelve days from 90 to 110 micrograms each of cortisone, compound F and two components more polar were excreted daily. Significantly increased excretion continued during sixteen post-treatment days. In the second Addisonian, free and conjugated corticoids were determined. During control periods from 0 to 20 micrograms of the four steroids found in the previous patient were excreted daily. On 100 mg. of cortisone daily each component increased to 100 to 500 micrograms daily. These findings suggest the reduction of C-11 and possibly C-3 ketones of cortisone at an extra-adrenal site.

56. The Inactivation of Cortisone by Tissue Slices.

Jean Louchart (by invitation) and *Joseph W. Jailer.*

College of Physicians and Surgeons, Columbia University, New York, N. Y.

The most important organ in the degradation of estrogens and androgens would appear to be the liver. However, since cortisone is so much more effective by mouth than are these steroids, the mechanism of its degradation is probably different. Therefore, it appeared to be of interest to determine which tissues are involved in cortisone inactivation. Accordingly, 100 to 250 micrograms of cortisone were incubated for three hours with approximately 300 mg. of tissue slices in 5 ml. of Krebs solution. The tissues were then homogenized and the resulting suspension injected into previously adrenalectomized male mice, and assayed according to a modification of the method of Venning, Kazmin and Bell (*Endocrinology* **38:** 79, 1946). Boiled tissue slices were employed as controls. Blood serum, and slices of muscle and brain demonstrated no significant inactivation of cortisone. Incubation with liver slices resulted in a 73 per cent inactivation in three hours. In addition, kidney and spleen slices were capable of inactivating cortisone (61 and 78 per cent respectively). These results are based on 36 experiments, each of which had 12 animals. The percentage of inactivation was calculated from the recovery obtained with boiled tissue slices run simultaneously. Experiments are under way to ascertain the factors which may modify the inactivation mechanism.

57. Incubation of Desoxycorticosterone and Other Adrenocortical Compounds With Various Surviving Tissues of the Rat.

John J. Schneider and *Patricia M. Horstmann* (introduced by W. M. Pearlman).

Department of Experimental Medicine, Jefferson Medical College, Philadelphia, Pa.

Methods have been devised for the recovery and determination of desoxycorticosterone and other adrenocortical compounds following incubation with various surviving tissues of the rat. Using ultraviolet spectroscopy as the analytic method, the incubation of desoxycorticosterone with surviving rat liver slices results in extensive loss of the conjugated unsaturated system. That this loss is reductive in nature follows from the isolation of significant amounts of allopregnane-3(β),21-diol-20-one. Rat liver brei, kidney slices and diaphragm segments are less active in this regard. Using periodate oxidation as a second analytic method, it has been shown that measurable lowering of the periodate titer occurs upon incubating desoxycorticosterone with liver and kidney tissue. The effect of various factors on these reactions has been studied. Data are presented contrasting the behavior of other adrenocortical compounds with desoxycorticosterone.

58. Influence of Adrenal Factors on Hemopoiesis in the Rat.

Albert S. Gordon, Sam J. Piliero (by invitation) and *David Landau* (by invitation).

Department of Biology, Washington Square College of Arts and Science, New York University, New York, N. Y.

Adrenalectomy in the rat results in a peripheral anemia which attains its maximum at approximately two to three weeks following the operation (Piliero, Landau and Gordon, *Science*, 1950). This anemia is macrocytic and normocytic in nature. Studies of the bone marrow of the salt-treated adrenalectomized rat reveal increases in the percentages of both neutrophilic and eosinophilic cells associated with a decrease in the concentrations of nucleated erythroid elements. Adrenal demedullation is without significant influence upon the peripheral blood or marrow picture. Daily administration of 2 cc. of an adrenal cortical extract concentrate (compound E equivalent—480 μg./cc.) or 0.5 cc. of Lipo-Adrenal Cortex (Upjohn) prevent successfully both the peripheral erythrocytic and the bone marrow alterations noted in the adrenalectomized animal. Compound E, in daily 3 mg. dosages, restores the bone marrow myelograms to normal values except for an accompanying marrow eosinopenia. Increased vacuolization is observed in the bone marrow sections of these animals. Daily administrations of compound E results in the development of the previously reported peripheral lymphopenia, eosinopenia and neutrophilia which are maintained for at least as long as two weeks in the adrenalectomized rat. Epinephrine (200 μg. daily) prevents the peripheral anemia and results in increases in the percentages of mature neutrophilic elements in the bone marrows of adrenalectomized rats. Marrow erythroid-myeloid cell ratios, however, are not affected by this agent.

59. The Salivary Na/K Ratio and Adrenal Salt-Regulating Factors: Prolonged Salt Retention With Desoxycorticosterone Trimethylacetate.

Thomas F. Frawley (by invitation) and *Peter H. Forsham.*

The Medical Clinic, Peter Bent Brigham Hospital and the Department of Medicine, Harvard Medical School, Boston, Mass.

The salivary Na/K ratio has been shown to vary inversely with the state of adrenal

cortical activity. It was found to be 1.3 (mean) in normal subjects, 5.0 (mean) in patient's with untreated Addison's disease and 0.5 (mean) in patients with Cushing's syndrome. With ACTH stimulation of the adrenal cortex there is a fall in the salivary Na/K ratio ranging from 18 to 68 per cent. The administration of 15 mg. of desoxycorticosterone acetate to normal subjects produces a 25 per cent fall in the salivary Na/K ratio. Changes in the salivary Na/K ratio follow alterations in the activity of the electrolyte-regulating steroids of the adrenal cortex.

The salivary Na/K ratio has been followed in 3 patients with Addison's disease treated with the trimethylacetate ester of desoxycorticosterone. Individual doses of the ester ranged from 30 mg. to 100 mg. intramuscularly. In 1 subject the salivary Na/K ratio fell from 2.07 to 0.8 in one week following a 60-mg. dose; it was 1.1 after four weeks and 1.6 after six weeks. The serum sodium showed no significant change. A single injection of desoxycorticosterone trimethylacetate has a prolonged effect of at least six weeks and acute signs of overdosage have not been observed.

60. Amino Acid Metabolism in Cushing's Syndrome and ACTH-Induced Hyperadrenocorticism, Using N-15 Incorporated in Glycine.

William Parson, K. R. Crispell (by invitation) and *Arthur Ebbert, Jr.* (by invitation).

Department of Internal Medicine, University of Virginia, Charlottesville, Va.

N-15 incorporated in glycine was fed during nitrogen balance studies to the following: (A) 6 normal men, (B) 3 normal men receiving ACTH, (C) four studies on 2 patients with Cushing's syndrome, and (D) 1 patient with Cushing's syndrome receiving testosterone.

The N-15 excretion rates were analyzed mathematically according to the techniques of Sprinson and Rittenberg (*J. Biol. Chem.* **180:** 715, 1949) and Hoberman (*Yale J. Biol & Med.* **22:** 341, 1950). Estimations were made of the "amino acid pool," the rate of tissue synthesis, and the conversion of amino acids to urea.

The following impressions were derived: Cushing's syndrome and ACTH administration are characterized by 1) an increased rate of conversion of amino acids to urea, 2) a decreased "amino acid pool," and 3) a decreased tissue synthesis. Testosterone tends to correct these abnormalities in Cushing's syndrome.

The degree of the alterations described above is such as to suggest that the increased conversion of amino acids to urea is the most significant of these changes.

61. Studies in Fat Metabolism: The Effect of ACTH and of Steroid Hormones Upon the Ketosis Induced by the Feeding of a Pure Fat Diet to Human Subjects.

Laurance W. Kinsell, Sheldon Margen, Lenore Boling (by invitation), *George D. Michaels* (by invitation), and *John W. Partridge* (by invitation), (with the technical assistance of Gerald Liebert and Judith Lange).

Institute for Metabolic Research, Highland County Hospital, Oakland, California.

In the course of evaluation of the effect of hormonal agents upon fat metabolism, patients have received a constant diet containing more than 2,000 calories derived from salad oil. The material has been made into an emulsion and administered at hourly intervals through a plastic tube, the tip of which is in the duodenum.

Clinical ketosis develops in those individuals who receive such a diet without other

treatment. The administration of ACTH in large dosage results in marked inhibition of the hyperketonemia, and ketonuria. Under these experimental conditions, cortisone and other steroids have failed to produce as great an antiketotic effect as has ACTH. This may be referable to inadequate dosage. Further evaluation of these agents is in progress.

These findings, together with long-term respiratory quotient studies, nitrogen balance data, and other observations in the same patients, further support the concept that some adrenal steroid or steroids, resulting from ACTH administration, produce changes in fatty acid metabolism characterized by increased rate of oxidation, decreased ketonemia and ketonuria, and increased blood sugar levels.

62. Remission in Cushing's Syndrome After Bilateral Hemiadrenalectomy.

Herbert S. Kuppermann, Arthur Bernstein (by invitation), *Anne Pappenheimer Forbes, Oliver Cope* and *Fuller Albright.*

Department of Therapeutics, N.Y.U.-Bellevue Medical Center, New York City; Departments of Endocrinology and Medicine, Newark Clinical Group, Newark, N. J.; Departments of Medicine and Surgery, Harvard Medical School; and Medical and Surgical Services, Massachusetts General Hospital, Boston, Mass.

There is no unanimity of opinion as to the most efficacious type of therapy to be employed in the treatment of Cushing's syndrome. X-ray therapy directed towards the pituitary gland has been widely used and has many advocates. However, many cases treated by x-ray do not experience permanent remission. Recently adrenal surgery has been proposed as an alternative but effective means of treatment of most cases of Cushing's syndrome. A typical case of Cushing's syndrome in a 17-year-old female showing all the classic clinical manifestations of the disease was treated by bilateral hemiadrenalectomy after failure of permanent remission to occur following radiation therapy to the pituitary gland. The disease has been in complete remission for the past two years following surgery. It is interesting to note that despite the prompt and striking clinical improvement noted after surgery, the adrenal cortices from both glands were perfectly normal from the histopathologic point of view. It is suggested that as soon as the diagnosis of Cushing's syndrome is established the status of the adrenal glands be determined by surgical laparotomy by observing one gland alone. If adrenal hyperplasia is noted, the contralateral gland may be expected to be large and resection should be done. If the gland is atrophied one may suspect a tumor in the contralateral gland. If the first adrenal is normal in size subtotal resection should be performed, and if no remission occurs the second adrenal should be explored. The primacy of the role of the pituitary gland or adrenal cortex in the pathogenesis of Cushing's syndrome is discussed.

63. Endocrine Studies Following Bilateral Complete Adrenalectomy in Man.

George W. Thorn, Thomas F. Frawley (by invitation), *Peter H. Forsham* and *D. Laurence Wilson* (by invitation).

Harvard Medical School and the Peter Bent Brigham Hospital, Boston, Mass.

Endocrine studies have been made on 5 patients following bilateral complete adrenalectomy, as an experimental approach to a study of the mechanisms involved in hypertensive vascular disease. In 3 patients both adrenals were removed at a single operation; in 2, a two-stage operation was performed. The patients were prepared preoperatively with ACTH and cortisone and carried successfully through the operation with cortisone,

aqueous adrenal cortical extract and neosynephrine. After complete adrenalectomy, administration of ACTH failed to cause a significant fall in circulating eosinophils or rise in urinary 17-ketosteroid or 11-oxysteroid excretion. In all patients a markedly negative sodium balance developed, which required the addition of 5 to 10 grams of supplementary sodium chloride or 0.5 to 1.0 mg. daily of desoxycorticosterone acetate, injected intramuscularly, in addition to 25 mg. of cortisone acetate daily administered orally in divided doses of 12.5 mg. On the above regimen, no evidence of adrenal insufficiency has become manifest (electroencephalogram, water test, or hypoglycemic episodes) except for increasing pigmentation in 3 patients. In all patients there was a reduction in 11-oxysteroid and 17-ketosteroid excretion.

2:00 P.M.—JOINT SESSION WITH THE AMERICAN DIABETES ASSOCIATION

DRS. E. H. RYNEARSON AND LESTER J. PALMER, PRESIDING

64. Experimental Diabetes Produced by Dehydroascorbic Acid.

Joseph V. Princiotto (introduced by Dr. Charles F. Morgan).

Georgetown University, Washington, D. C.

Rabbits given intravenous injections of dehydroascorbic acid (DHA), in 4 equal doses at 15-minute intervals totalling .60 Gm./Kg. following twelve to twenty-four hours of fasting produced what appears to be a pancreatic experimental diabetes.

Of the 48 rabbits treated with DHA over the past two years, 44 became persistently diabetic as indicated by polyuria, glycosuria and hyperglycemia. The rabbits were considered diabetic if the blood sugar values were greater than 200 mg. per cent. In the animals that survived, the blood sugar changes following intravenous injections of DHA are the same as seen in alloxan diabetes, that is: a transient hyperglycemia; a more or less severe, and, in some animals, fatal hypoglycemia; and a secondary hyperglycemia. Six animals showed minute capillary bleeding in the retina. Two others showed unilateral cataracts.

Our attempts to produce diabetes with 2,3-diketogulonic acid have been entirely unsuccessful to date.

Intravenous injection of cysteine (7.5 mM/Kg.) or glutathione (3.0 mM/Kg.) one minute prior to the injection of a diabetogenic dose of DHA protected the rabbits from diabetes.

Our results are similar to the diabetogenic effects of vitamin C derivatives in another species, the rat, as reported by J. W. Patterson (*J. Biol. Chem.* **183:** 81, 1950) and J. W. Patterson and A. Lazarow (*J. Biol. Chem.* **186:** 141, 1950).

65. Changes in Serum Potassium During the Intravenous Glucose Tolerance Test.

D. Laurence Wilson (by invitation) and *George W. Thorn.*

Harvard Medical School and the Peter Bent Brigham Hospital, Boston, Mass.

Serial estimations of blood sugar and serum potassium were performed in human subjects following the intravenous infusion of glucose, 0.5 Gm./Kg., over a thirty-minute period. In 22 nondiabetic subjects, the maximum fall in serum potassium during the tests averaged 0.2 mEq./L, with a standard error of 0.04. In 9 patients with uncomplicated diabetes mellitus, the mean maximum fall was significantly greater (0.4 ± 0.13); in addition, 2 patients with anatomic pancreatic disease without hyperglycemia showed

maximum falls of 0.7 and 0.9 mEq./L. In contrast, in 9 patients with diabetes plus liver disease, this fall was 0.1 mEq./L ± 0.08. Thus, the exaggerated potassium fall characteristic of the diabetic state is abolished by concomitant liver disease. In 3 patients with uncomplicated liver disease, augmented falls in serum potassium (0.6–0.7 mEq./L) were found. Two patients with severe skeletal muscular atrophy showed maximum potassium falls of 0.5 and 0.8 mEq./L. An exaggerated potassium fall thus appears to occur in the presence of impairment of either muscle or liver utilization of glucose, but not when both are impaired.

66. The Effect of Cortisone on the Blood Glutathione Level.
Arnold Lazarow.

Western Reserve University School of Medicine, Cleveland, Ohio.

67. Amino Acids in Experimental Diabetes.
Smith Freeman.

Northwestern University Medical School, Chicago, Ill.

68. The Banting Lecture: The Endocrine Control of the Blood Sugar.
C. N. H. Long.

Yale University Medical School, New Haven, Conn.

69. Hyperinsulinism.
Penn G. Skillern (by invitation) and *E. H. Rynearson.*

Mayo Clinic, Rochester, Minnesota.

The clinical features of 65 patients with functioning islet cell tumors of the pancreas and 6 patients with hyperinsulinism due to surreptitious self-administration of insulin are discussed. No patients with hyperinsulinism due to hyperplasia of the islet cells were encountered. The pancreas of 1 patient who was thought to have hyperplasia of the islet cells was found to have, on re-examination, an adenoma 2 mm. in diameter. One patient was explored unsuccessfully four times in sixteen years for islet cell adenoma. At post mortem, an islet cell adenoma was found. Only the 48-hour fast has been of help to us as a diagnostic test. Only 1 of 47 patients with functioning islet cell tumors failed to have a hypoglycemic response during the 48-hour fast. The symptoms of functioning islet cell tumors may be very difficult to distinguish from those due to surreptitious self-administration of insulin. All of the 6 patients in this latter group were either nurses or diabetic patients. The diabetic patient whose diabetes disappears and is replaced by hypoglycemic attacks should be suspected of having this syndrome rather than a functioning islet cell tumor.

TO BE READ BY TITLE

70. Effect of Acute Stress Upon Blood Constituents, White Cells, and Urine Constituents in Normal Individuals.
W. James Kuhl, Jr. (by invitation) and *Elaine P. Ralli.*

Department of Medicine, College of Medicine, New York University-Bellevue Medical Center, New York, N. Y.

Fourteen normal males were immersed in water at 9.5° C. for a period of eight minutes. The acute changes were followed with blood and urine samples taken prior to the im-

mersion, immediately following and at one, two and four hours after immersion. At these intervals the temperature, blood pressure, heart rate, total WBC, neutrophils, lymphocytes, eosinophils, serum sodium and chloride, plasma potassium, total proteins, serum water, blood sugar, ascorbic acid, serum cholesterol and esters, and excretion of sodium, potassium, chloride, uric acid and creatinine were followed.

The statistically significant changes which occurred and the time of their occurrence after immersion were as follows: 1. A decrease in temperature (immediate, one and two hours); 2. An increase in systolic and diastolic blood pressure (immediate) and a decrease (two hours); 3. A decrease in heart rate (one, two and four hours); 4. An increase in neutrophils (immediate); 5. A decrease in lymphocytes (two hours); 6. A decrease in eosinophils (two hours); 7. A decrease in serum chloride (immediate); 8. An increase in total proteins (immediate and four hours); 9. A decrease in serum water (immediate); 10. An increase in total cholesterol (four hours); 11. An increase in the urine uric acid/creatinine ratio (two and four hours).

Although no single test of adrenal cortical activity showed a uniformly positive result, the above changes were significant following this acute stress situation.

71. The Circulating Eosinophil Response of Mongoloids to Epinephrine.

Irving Rothchild and *John Patrick Riepenhoff* (by invitation).

Departments of Obstetrics and Gynecology, and Pediatrics, Ohio State University, Columbus, Ohio.

Mongolism has been described as a pituitary disorder (C. E. Benda: *Mongolism and Cretinism*, Grune and Stratton, 1946). To test at least the possibility of pituitary-adrenal cortex insufficiency, the circulating eosinophil response to epinephrine has been studied in mongoloid subjects at the Columbus State School. Adrenalin chloride (1/1,000) injections were made subcutaneously, at about 8:00 a.m., in fasting subjects. Total eosinophil counts, using the method described by Thorn, were used. The 0 time, and four-hour counts were made from duplicates, and the one, two and three-hour counts from single finger blood samples. Of 12 mongoloids given 0.20 cc., 8 responded with decreases of 50 per cent or more, and 4 with decreases of between 25 per cent and 50 per cent in the circulating eosinophil count by the fourth hour after injection. Sex or age differences were not obvious. No response to 0.10 cc. was seen in 3 mongoloid girls. Two out of 4 normal children of comparable ages showed no decrease; 1, only a partial decrease; and 1, more than a 50 per cent decrease in the eosinophil level in response to 0.10 cc. Two normal children and 2 cretin girls responded to 0.20 cc. with decreases of 50 per cent or more by the fourth hour after injection. It may be tentatively concluded that the mongoloid does not show any striking deviations from normal in at least this test of pituitary-adrenal cortex function.

72. Inhibition of the Adrenalinic Eosinopenia Due to Thiouracil Administration in Hyperthyroid and Normal Human Subjects.

F. A. de la Balze, R. E. Mancini (by invitation), *C. J. Scarpa* (by invitation) and *F. C. Arrillaga* (by invitation).

IIIa Catedra de Clinica Médica, Facultad de Ciencias Médicas, Buenos Aires, Republica Argentina.

The variations of the adrenalinic eosinopenia during the administration of thiouracil were studied in 9 euthyroid and in 19 hyperthyroid human subjects. Eosinophil counts

were carried out, using the Dunger method; and the adrenaline test, after Forsham and Thorn. Duration of treatment and observation on adrenalinic eosinopenia varied from sixty-five to three hundred and thirty days. As a comparison, variations in adrenalinic eosinopenia were studied during one hundred days in 20 untreated normal subjects. The most significant modification observed consisted in a manifest and maintained inhibition of the adrenalinic eosinopenia which appeared during the treatment in 9 hyperthyroid and in 4 euthyroid subjects. When thiouracil was withdrawn, the adrenalinic eosinopenia returned to normal. In 7 of the patients with the mentioned inhibition, the four-hour ACTH test was applied; 3 of them showed a normal eosinophil response, 3 a subnormal and 1 a conterminus response. Our results lead us to believe that the adrenocortical changes are the consequence of an insufficiency of the adenohypophysis.

73. Turner's Syndrome Accompanied by Mongolism: Multiple Degenerative State.

Manuel M. Villaverde.

Habana, Cuba.

Two cases are recorded. In both patients the diagnosis had been incomplete when they consulted us for the first time. Case 1, pituitary microsomia; Case 2, mongoloid idiocy. Both showed intense mongoloid traits and oligophrenia.

The diagnosis of Turner's syndrome is based on: 1) dwarfism; 2) short neck with low implantation of hair; 3) stocky build and shield-like chest; 4) lack of sexual development; and 5) minor symptoms.

Mongolism is based on: 1) Chinese-like face; 2) intense epicanthus; 3) prognathism; 4) interest in music and other psychic traits; 5) minor symptoms.

We did not find similar records in the literature, although there may be some, such as seems to be Case 1 in Greenblatt and Nieburgs' report.

These cases stress the importance of congenital anomalies in the integration of the syndrome and help us to consider its fundamental traits as a consequence of some genetic degenerative state which is characterized by the presence of multiple congenital anomalies, among them, and of paramount importance, the lack of sexual development.

74. Adipose Gynandrism and Gynism.

S. Leonard Simpson.

London, England.

This is presented as a new clinical syndrome or concept, although fragments of it have appeared from time to time in the literature.

Gynandrism is applied to the male as indicating a mixture of male and female characteristics and gynism to the female as suggesting an intensification of female qualities. The syndrome presents itself usually between the ages of 10 and 16, but indications may be present from infancy or early childhood.

It might be regarded as a variation of normal puberty but only when present in mild degree and then only in so far as major endocrine manifestations of pregnancy and the climacteric might be so regarded.

The chief features are adiposity involving face and trunk, high color sometimes amounting to plethora, height some two inches above the average, weight some twice the average, advanced bone age, early union of epiphyses in the female, early menarche but delayed puberty in the male, wide female type of pelvis in both sexes, large breasts in

females and not infrequently in males, female-type horizontal pubic hair in males (but not invariably), female facies and attitudes in many males, red or purple lineae distensae on the lower abdomen, buttocks, or lumbar regions and sometimes on the breasts and flanks. It may be associated with hypertension, albuminuria and polycythemia.

It is suggested that the disorder is a pituitary-adrenal overactivity and that the adrenal cortex is secreting an excess of compound E and compound A and of estrogens and that there is excessive formation and deposition of fat from carbohydrate.

Regardless of the hormonal theories of etiology, there appears no doubt that we are dealing with a definite clinical entity, even if mild degrees are classified as constitutional and with the limits of normality.

75. The Hypometabolic State: A Clinical Entity.

B. A. Watson.

Metabolic Service of the Clifton Springs Sanitarium and Clinic, Clifton Springs, N. Y.

A "twilight zone" exists between an individual with a normal metabolism and one with true hypothyroidism or myxedema which can easily be overlooked. Patients frequently consult a physician because of fatigue for which no organic basis can be demonstrated. Clinically one should suspect the presence of the hypometabolic state if an affirmative answer is obtained to three or more of the following questions: 1) morning fatigue and "evening pep"? 2) cold sensitivity? 3) brittle nails? 4) splitting hair? 5) coarse dry skin? 6) lack of perspiration? 7) menstrual irregularities? The clinical diagnosis can be confirmed further if a low wakening temperature, bradycardia and a BMR below or at the lower limits of normal is found. This paper deals with a number of clinical cases treated by means of desiccated thyroid with good results. Treatment consisted of giving 2 to 4 grains of thyroid daily in divided doses, after breakfast and lunch. Patients were warned not to expect therapeutic results under ten to fourteen days. If improvement was noted in two to three days. it was felt that it was on purely psychologic grounds. Indications and contraindications to thyroid therapy in this condition are enumerated.

76. Influence of Growth-Promoting Factors on Some Liver Constituents Following Partial Hepatectomy in Rats.

Mary E. Dumm, and *Elaine P. Ralli.*

Department of Medicine, College of Medicine, New York University-Bellevue Medical Center, New York, N. Y.

Adult male rats were placed on a 38 per cent fat, 9 per cent protein diet. After six weeks a partial hepatectomy was done, removing about 70 per cent of the liver. The partial hepatectomies were done through a dorsal incision and the technique appreciably decreased postoperative mortality. The liver was analyzed for solids, lipids, nitrogen and chloride. After operation some rats were continued on the high fat diet; others were transferred to a low fat-high protein or to a low fat-high carbohydrate diet. On all diets the influence of additional growth-promoting factors was tested by injecting half the rats with the substance being tested, and the remaining animals with saline. The animals were sacrificed at varying intervals after hepatectomy, the remaining liver removed, and analyzed. Sections of liver were prepared for pathologic study both at operation and

at sacrifice. Rats continued on high-fat diet and injected with saline after partial hepatectomy showed an increase in liver fat, both histologically and chemically; in rats injected with liver extracts or vitamin B_{12} the liver fat decreased. Rats fed the high-protein diet after partial hepatectomy showed a decrease in lipid which was not dependent on the substance injected.

77. The Place of Anorexic Agents in Weight Reduction.

E. Philip Gelvin and *Thomas H. McGavack.*

Department of Medicine, New York Medical College, New York, N. Y.

The foundation of a successful weight-reduction regimen is a satisfactory low caloric diet. Such a diet will frequently cause discomfort to an obese individual, leading to discouragement and abandonment of the regimen. A suitable anorexic agent will tide the patient over this initial period in a great many cases.

At the Obesity Clinic of the Welfare Island Dispensary, New York, several anorexic agents were tried. r-Amphetamine and d-amphetamine were given to 30 patients without any dietary restrictions. An initial early loss of weight occurred in 27, 14 of whom maintained an average loss of 1 pound or more a week for eight weeks. This loss of weight, however, gradually tapered off and after twenty weeks the rate of weight loss was about half a pound per week. Side effects were few, and consisted of symptoms of central nervous system stimulation.

A preparation containing 30 mg. of vitamin C was given to 15 patients, while similarly sized groups were given a placebo and no medication, respectively. All groups were on the same 1,000 caloric diet. Apparently vitamin C had little or no effect.

A new preparation "Aptrol" (p-methylamphetamine sulfate) was tried in a group of 65 patients, 48 of whom served as their own control, *i.e.*, were observed for a period while taking the medication, and another period while taking a placebo with a similar appearance. All patients were kept on a 1,000 calorie diet. While on the medication, patients did four times as well as when on placebo. The incidence of side reaction was similar to that found with the amphetamines, and consisted of dizziness, an "all gone" feeling, and minor gastro-intestinal complaints.

In the treatment of obesity, the primary importance of the diet must be explained to the patient, lest the secondary value of the anorexic agent be over-emphasized. As soon as it is felt that the patient is adjusted to the new dietary regimen, the anorexic agent is gradually withdrawn, to prevent the patient's forming a psychologic attachment to the medication.

78. Acromegaly—Case Presentation: Hormonal and Radiation Therapy.

Rita S. Finkler and *George M. Cohn.*

Newark Beth Israel Hospital, Newark, N. J.

A case of active acromegaly, which received both hormonal and radiation therapy, is presented. The presence of a pituitary tumor was indicated by the results of repeated radiologic examinations, visual fields and hormonal assays of pituitary gonadotropins and 17-ketosteroids. There were marked enlargement and erosion of the sella turcica; decreased pituitary gonadotropins and increased 17-ketosteroids. The findings of blood glucose and insulin tolerance tests, and serum phosphorus levels were characteristic for pituitary adenoma. Hormonal therapy, consisting of combined estrogens and androgens,

controlled the symptoms for a period of one and a half years but did not prevent a resurgence of pituitary overactivity. The patient was then given inhibiting doses of radiation to the pituitary region with subsequent improvement in symptoms and control of the pituitary hyperactivity. The supplementation of therapy, by radiation, augmented and prolonged the primary beneficial responses procured by hormonal therapy. Parallel favorable changes were observed in the biochemical studies.

79. Influence of Thyrotropic Hormone (TSH) and Thiouracil on the Oxygen Consumption of the Guinea Pig.

Elizabeth A. Herrold (by invitation), *Roy K. Rinehart* (by invitation), *Harold E. Coombs* (by invitation) and *James H. Leathem.*

Division of Macrobiology, Research Department, Ciba Pharmaceutical Products, Inc., Summit, N. J.

A test animal which simulated the hyperthyroid patient was deemed desirable for testing thiouracil. One chick unit per day of TSH (Parke Davis) usually raised the oxygen consumption of 250-gram guinea pigs 30 to 50 per cent above that of untreated controls within ten days. Despite continued TSH treatment, oxygen consumption declined gradually and was recorded at 15 per cent above control levels on day 20. Daily dosages of 0.5 and 2.0 units of TSH approached the 1.0 unit response in degree but not in duration. The daily administration of 100 mg. of thiouracil by stomach tube to TSH-treated animals was studied to determine if a more rapid decline in oxygen consumption would be effected by an antithyroid agent. Thiouracil was administered to these guinea pigs from days 6 to 20. TSH administration alone, for ten days only, was followed by a rapid decrease in oxygen consumption on days 15 and 20; thiouracil did not hasten the decrease. Other groups were treated with 1 unit of TSH, 100 mg. of thiouracil, and the combination, for twelve days. Oxygen consumption was increased 25 per cent and 27 per cent by TSH after five and twelve days respectively. Thiouracil in combination with TSH permitted increases of 19 per cent and 7 per cent, whereas thiouracil alone did not appreciably lower the oxygen consumption.

80. The Use of Thyrotropic Hormone (TSH) in the Diagnosis of Myxedema.

Lester M. Levy (by invitation), *Agamemnon Despopoulos* (by invitation) and *William H. Perloff.*

The Endocrine Clinics, Philadelphia General Hospital, Philadelphia, Pa.

The use of thyrotropic hormone in conjunction with a radioiodine tracer test has proven valuable in the differential diagnosis of pituitary versus primary thyroid myxedema. The 24-hour uptake of radioiodine is measured. Two to four weeks later, three consecutive daily intramuscular injections of 20 to 30 mg. of thyrotropic hormone (Armour) are administered. The tracer dose of I^{131} is given the fourth day, and the uptake is measured twenty-four hours later.

Normal individuals show a marked increase in uptake. Primary hypothyroid patients maintain a low radioiodine accumulation similar to their control values. Pituitary hypothyroid patients resemble normal cases in having a great increase in I^{131} uptake. The value of this test is demonstrated by a series of patients in whom the differential diagnosis could not be made by other means.

81. Blood Radioiodine Concentration and Blood Radiation Dosage During I^{131} Therapy for Metastatic Thyroid Carcinoma.

S. M. Seidlin, A. Aaron Yalow (by invitation) and *Edward Siegel* (by invitation).

Montefiore Hospital, New York, N. Y.

To determine total body radiation dosage, blood radioiodine concentration was studied following 73 therapeutic doses of I^{131} to 24 patients with metastatic thyroid carcinoma. Following I^{131} ingestion, weighed samples were taken for periods up to thirty-five days in order to determine radioiodine concentration (μc/Gm. of whole blood). The specific radiation dose (S.R.D.) in rep/100 mc depends on both the mass and function of thyroid tissue, non-neoplastic and neoplastic. For the first two days following a therapeutic dose, the S.R.D. is significantly lower for patients with a thyroid gland than for thyroidectomized patients. Subsequently, the situation tends to be reversed. During this latter period, the S.R.D. is higher for patients having functioning metastases. However, *total* S.R.D. appears to be independent of the presence of the thyroid gland. The S.R.D. has varied from 25 to 228 rep/100 mc of I^{131} administered. Total body radiation up to 400 rep has been delivered after a single dose of I^*, occasionally producing temporary leucopenia. Following radiation-thyroidectomy doses and following the administration of 25 per cent of the therapeutic doses to thyroidectomized patients with functioning metastases, a characteristic irradiation produced a rise as has been observed in the concentration of I^{131} in the blood, usually four to eight days after the dose.

82. Incapacitating Masked Hyperthyroidism Successfully Treated With the Imidazoles.

Jacqueline Chevalley and *Thomas H. McGavack.*

New York Medical College, Metropolitan Hospital Research Unit, New York, N. Y.

Fifteen cases of patients with a history of chronic disease over periods of one to twenty years are presented because of the existence of a masked hyperthyroidism, either responsible alone for the patient's complaints or aggravating other conditions. Marked improvement of the general condition was obtained after control of the hyperthyroidism.

Advantages of treatment with the new antithyroid preparations: 1 methyl-2-mercaptoimidazole (Tapazole), 1,ethyl-2-mercaptoimidazole and 1-n-propyl-2-mercaptoimidazole are presented.

83. Chronic Thyrotoxic Myopathy.

Edward L. Quinn (by invitation) and *Richard L. Worcester* (by invitation) (introduced by J. P. Pratt).

Henry Ford Hospital, Detroit, Mich.

Muscular weakness and atrophy are occasionally the predominant features of the thyrotoxic state. If in addition personality aberration, nervous irritability, and loss of libido severe enough to threaten the patient's domestic status are present, and only minimal symptoms and signs usually associated with thyrotoxicosis exist, the latter may be easily overlooked. Furthermore, marked abnormality in creatine-creatinine metabolism and steroid hormone deficiency are found and correction of the hyperthyroidism may result in only partial alleviation of the syndrome. Supplemental steroid hormone therapy may be required for complete control. An illustrative case is presented.

84. Refractoriness to Antitetanic Therapy: Occurrence in a Case of Surgical Hypoparathyroidism.

Raymond W. Blohm, Jr. and *Otto A. Wurl* (introduced by Roberto F. Escamilla).

Letterman Army Hospital, San Francisco, Calif.

The patient is a 30-year-old housewife first hospitalized because of symptoms of hyperthyroidism. Examination and study showed typical exophthalmic goiter; and after preparation with propylthiouracil and Lugol's solution, subtotal thyroidectomy was performed, with the procedure proceeding smoothly. Thirty-six hours later carpopedal spasm occurred. One parathyroid gland was found in the excised tissue. Treatment with diet, dihydrotachysterol, vitamin D, calcium and amphojel seemed effective for two weeks, but then control was lost in spite of increasing doses of medications, including 18 cc. of dihydrotachysterol daily. Other complications were progressive exophthalmos, mild hypothyroidism, and temporary paralysis of the left vocal cord. Latent tetany continued with occasional episodes of severe tetany controlled by intravenous calcium. Metabolic studies were then carried out during a four months' period of hospitalization. Parathormone and again dihydrotachysterol were given during this period. There was a consistent positive calcium balance during these studies. The patient still has latent tetany despite a regimen which includes a dairy-free diet, calcium orally, amphojel, and 500,000 units of vitamin D daily.

85. The Antagonistic Effect of Various Steroids to Testosterone and Estrone-Induced Changes in the Castrate Rodent.

F. C. Dohan, Arden F. Cordray (by invitation) and *V. S. Palladino* (by invitation).

University of Pennsylvania, Philadelphia, Pa.

The castrate female mouse has been used to test the effect of various steroids upon the estrone-induced weight and cellular changes in the vagina (plus cervix), and uterus. Testosterone propionate, desoxycorticosterone, progesterone, 6-dehydroprogesterone, 16-dehydroprogesterone, and etiocholane 3-one-17-acetoxy have been found to decrease the estrone-induced weight increase of the vagina (plus cervix). Effects on the vaginal smear have also been found. The castrate male mouse receiving testosterone propionate has been used to test the effect of various steroids on the weight and cellular changes in the seminal vesicle. Desoxycorticosterone, progesterone, and 6-dehydroprogesterone antagonize the testosterone-induced increase in weight in this organ. In addition, progesterone antagonizes the weight increase produced by 0.25 mg. of testosterone propionate in the prostate, as well as the seminal vesicle of the castrate rat. We have not yet tested these compounds for possible contaminants. However, it is concluded that the antagonistic effects of these compounds are not associated with any single major physiologic activity (such as androgenic or progestational). Furthermore, it seems probable that antagonistic effects may be expected from steroids with no other major physiologic activity.

86. The Relative Effectiveness of Different Androgenic Preparations on the 17-Ketosteroid Excretion in Man and Gonadotropic Inhibition and Growth-Stimulating Effects on the Male Accessory Glands in the Rat.

Herbert S. Kupperman, Betty Silver (by invitation) and *Ralph Postiglione* (by invitation).

Department of Therapeutics, New York University-Bellevue Medical Center, New York, N. Y.

Three objective criteria were employed to study the comparative efficacy of testosterone, testosterone propionate and methyl androstenediol with respect to their effect and duration of action. The study included an evaluation of the type of vehicle and mode of administration upon the response to the steroid preparations. The procedures employed included:

(a) Determination of the 17-ketosteroid excretion (utilizing the Zimmermann reaction) on a series of hospitalized patients, before, during, and after administration of the androgenic preparation. In all cases during the experimental period the patient received a daily dose of 50 mg. of each steroid for fourteen days.

(b) Inhibition of gonadotropic hypersecretion in the castrate partner of parabiotic rats, and

(c) Growth-stimulating effects on the seminal vesicle and prostate glands of castrate rats autopsied from two to three days up to thirty days after a single 10-mg. dose of the steroid.

No measurable increase in 17-ketosteroid excretion over control values was observed following the use of testosterone propionate in suppository form, or from either oral or intramuscular administration of methyl androstenediol. However, comparable and marked elevations in 17-ketosteroid excretion were noted following oral as well as intramuscular dosage of both testosterone and its ester, regardless of the type of vehicle employed.

In marked contrast to the results of the metabolic excretion studies, the parenteral preparations of testosterone and testosterone propionate were found to vary markedly in biologic activity in the animal experiments. Testosterone propionate in oil was found most effective in inhibiting the gonadotropic hypersecretion in parabiotic rats, whereas testosterone propionate as aqueous suspension or testosterone in an aluminum phosphate aqueous suspension were most effective in inducing and maintaining growth of the accessory glands of the castrate male rat. Testosterone in oil was least effective. The discrepancy between the relatively uniform excretion studies and the variable biologic response to the testosterone and its ester in different vehicles is discussed.

87. Effect of Ascorbic Acid on the Connective Tissue Reaction Induced in Guinea Pigs by Estrogen.

W. v. B. Robertson, F. W. Dunihue and *E. C. Sanborn* (introduced by W. Raab).

College of Medicine, University of Vermont, Burlington, Vt.

Young male guinea pigs when eating a stock diet and injected with 0.5 mg. of estradiol benzoate twice weekly showed striking changes in mesenchymal tissues within two to four weeks. Massive mucinous edema of the dermal and subcutaneous connective tissue which extended into the deep fascia was especially prominent in dependent parts of the body. When examined microscopically this tissue showed many areas of intense fibroblastic activity. Scattered areas of free red cells were seen in this tissue. The joints were swollen and the legs and forepaws were often partially or completely immobilized by tissue masses in the axillary or inguinal regions. If the stock diet, which assayed *ca.* 25 mg. of ascorbic acid per 100 grams was supplemented with 50 mg. of ascorbic acid every other day, these changes could be prevented. When fed an ascorbic acid-free diet, similar changes occurred but the animals died before all of the changes developed. The

effect on this reaction of varying estrogen dosage, ascorbic acid dosage, stage of growth of the guinea pigs, of other steroids and of nonspecific stress have been investigated. These results which are reported, when considered in the light of previous work, suggest that this is not simply a nonspecific stress reaction, nor is it acute scurvy provoked by estrogen. The possibility is discussed that we are dealing with an example of the fibrogenic activity of estradiol as modified by suboptimal levels of ascorbic acid.

88. The Effect of Daily Injections of Progesterone on the Persistent Estrus Produced in Rats by Hypothalamic Lesions.

Monte A. Greer.

New England Center Hospital and the Department of Medicine, Tufts College Medical School, Boston, Mass.

Persistent estrus was produced in rats with fairly regular consistency by small bilateral electrolytic lesions in the lower anterior hypothalamus. These lesions were located in or near the ventromedian nucleus but apparently were not identical with those producing obesity. Some of the animals became obese without exhibiting persistent estrus, some entered persistent estrus without becoming obese, and some showed both phenomena. The estrus pattern following operation varied from animal to animal. Almost always there was an eight- to twelve-day period of diestrous vaginal smears immediately following operation. Some of the animals then maintained continuous vaginal cornification while others first had a few regular cycles, following which they entered persistent estrus. Occasionally one or two normal cycles would be interspersed.

A single injection of 0.5 mg. of progesterone resulted in a diestrous smear in twenty-four to forty-eight hours followed by a return to persistent estrus seventy-two hours after the injection, as found by Everett (*Endocrinology* **32**: 285, 1943) in a persistent-estrus strain of rats. Daily injections of 0.5 mg. of urogesterone caused the resumption of normal cyclic vaginal changes. Upon cessation of therapy persistent estrus usually returned within one to four days. Occasionally, however, evidence of cyclic activity continued.

89. Induction of Cystic Endometrial Hyperplasia in the Hamster With Estradiol; Prevention and Treatment with Progesterone.

A. E. Rakoff, Mona L. Gross (by invitation) and *B. L. Wellenbach* (by invitation).

Jefferson Medical College and Hospital, Philadelphia, Pa.

Continued administration of estradiol to oophorectomized adult golden hamsters caused a marked "cystic" hyperplasia of the endometrium, characterized by the presence of numerous large dilated tubular glands lined by low epithelium and surrounded by small closely packed stromal cells. This pattern appeared quite regularly within four weeks with the subcutaneous injection of an aqueous suspension of estradiol crystals, 100 micrograms once weekly, and within two or three weeks when the dose was 200 micrograms weekly. These changes were preceded by simple hyperplasia, evidenced by smaller glands lined by tall columnar epithelium, with piling up of cells and numerous mitoses. Almost complete regression occurred within four weeks after discontinuation of injections, but more rapidly if progesterone was given (3 mg. in aqueous suspension). The simultaneous administration of 10 mg. of an aqueous suspension of crystalline progesterone with each injection of estradiol almost completely prevented the hyper-

plastic endometrial changes but produced a marked decidual-like reaction of the stroma and sometimes the appearance of an exudate containing round cells. Similar findings were noted after more prolonged administration of both hormones (total of 1,000 gamma of estradiol and 100 mg. of progesterone in ten weeks).

90. The Role of the Pituitary and Steroid Hormones in Growth Changes of the Cervix.

H. E. Nieburgs and *Lane H. Allen* (by invitation).

Department of Clinical Cytology, Medical College of Georgia, Augusta, Ga.

The detection of about 300 cases of cervical cancer in the noninvasive and invasive phases suggested that cancer in its incipiency is associated with prolonged estrogen activity and apparently also increased pituitary activity.

Five series of animal studies were carried out to induce this condition experimentally. In the first two series the effect of estrogen with and without additional thiouracil was studied in order to determine the effect of increased basophil-cell activity of the pituitary on cervical epithelium in conjunction with prolonged estrogen administration. In the third series the effect of thyroid hormone administration in addition to stilbestrol was investigated. These experiments were repeated in a fourth series of hypophysectomized animals. Vaginal smears were taken periodically and it was found that the addition of thiouracil to stilbestrol inhibited the production of an estrous smear. Sections of the cervix and uterine horns showed decreased epithelial proliferation in animals given thiouracil in addition to stilbestrol. Such changes did not occur in the hypophysectomized animals. In a fifth series of experiments, cervical tissue comprising the squamous and columnar epithelium was transplanted into the spleen next to ovarian tissue in ovariectomized animals. This was carried out to produce simultaneously both increased pituitary activity and prolonged estrogenic stimulation from the adjacent ovarian tissue.

Histologic changes of glands and tissues are presented and discussed.

91. Some Hormone Studies During Normal and Toxemic Pregnancy.

C. W. Lloyd, E. C. Hughes (by invitation), *J. Lobotsky* (by invitation) and *J. Rienzo* (by invitation).

Department of Obstetrics, State University of New York, Medical Center at Syracuse, N. Y.

Serum antidiuretic activity, urinary total, freely-water-soluble, and poorly-water-soluble corticosteroid, pregnanediol and chorionic gonadotropin were studied throughout 6 normal pregnancies and in 11 cases of pre-eclampsia. No significant difference was found between the antidiuretic activity during any of the three trimesters of normal pregnancy or between the average activity of the patients during the third trimester and the toxemic patients. During the third trimester urinary total corticosteroid averaged 60 per cent higher, freely-water-soluble corticosteroid 40 per cent higher, and poorly-water-soluble corticosteroid 112 per cent higher than during the first trimester. The total corticosteroid excretion of the 5 patients with severe toxemia was more than 80 per cent greater than normal patients in the same trimester and poorly-water-soluble material was more than 100 per cent greater than in the normal. Pregnanediol and chorionic gonadotropin followed the pattern described by other authors, with the pregnanediol increasing throughout normal pregnancy and the chorionic gonadotropin high during the first trimester but low thereafter. In the patients with severe toxemia, pregnanediol was 30 per cent lower and chorionic gonadotropin was 530 per cent greater than in normal patients. In less severe toxemia, all of the changes were less marked.

92. Effects of Testosterone Propionate on Human Ovulation.

Edwin C. Jungck, Willis E. Brown and *Byron A. Bennett* (by invitation).

University of Arkansas School of Medicine and the Arkansas State Hospital for Nervous Diseases, Little Rock, Ark.

In 1948, Brown, Bradbury and Jennings found that estrogen given early in the menstrual cycle might delay ovulation, but did not prevent it. If, on the other hand, estrogen were given in increasing dosage throughout the cycle, ovulation was prevented. Estrogen given in the postovulatory interval did not affect the length of the cycle or the life span of the corpus luteum.

In order to study the effect of testosterone propionate upon ovulation and the menstrual cycle, 6 women whose ovarian activity had been followed for one year previously by weekly endometrial biopsies and who had regular, ovulatory menstrual cycles were given graded doses of testosterone propionate at varying intervals in the cycle. The effect of this treatment was determined by further weekly endometrial biopsies.

Five milligrams of testosterone propionate given intramuscularly daily for the first fourteen days of the cycle caused no change in the length or character of the cycles. Ten milligrams given intramuscularly daily for the first fourteen days caused a two-week prolongation of the follicular phase of one of the six cycles, but all cycles were ovulatory. Fifteen milligrams given intramuscularly daily for the first fourteen days caused a two-week prolongation of four of the six cycles, and two of the four prolonged cycles were anovulatory.

Additional studies are under way on administration of the hormone at midcycle and during the postovulatory interval, and will be reported.

93. Dysmenorrhea—Its Effective Control by Preovulatory Administration of Methyl Testosterone or Androstenediol Without Inhibition of Ovulation.

Stanley J. Goodman (by invitation) and *Herbert S. Kupperman.*

Departments of Gynecology and Endocrinology, Newark Clinical Group, Newark, N. J.

Since functional dysmenorrhea has been noted invariably in menstrual periods associated with a secretory endometrium, modern therapy of painful menses has been based upon inhibition of ovulation by the preovulatory administration of stilbestrol or other estrogens. Dysmenorrhea may be successfully controlled by this method, providing ovulation is repeatedly inhibited during each menstrual cycle. Due to the inherent disadvantages of this method in the therapy of dysmenorrhea and its interdicted use in sterility patients, preovulatory administration of methyl testosterone or androstenediol was employed in this study. Methyl testosterone was administered to 50 patients in 10-mg. doses given three times a day, starting four or five days preceding the expected day of ovulation and continued for six to eight days. Methyl androstenediol was prescribed according to a similar time schedule but in single 25–50 mg. doses per day. Both preparations were therapeutically effective and comparable in action. The preovulatory use of ethynyl testosterone, on the other hand, did not prove to be effective in controlling dysmenorrhea. The advantage of methyl testosterone or androstenediol over the use of estrogens is that ovulation is not inhibited despite the attainment of relief of menstrual pain. This is supported by the fact that secretory endometria, ovulatory rises in body temperature and several pregnancies were noted after the preovulatory administrations of the androgens in the doses employed. Methyl androstenediol, perhaps, has the added

advantage in that arrhenomimetic phenomena are improbable following its use in the previously mentioned dose levels. Both preparations proved to be effective when used in successive and repeated cycles. The possible mechanism involved in the control of dysmenorrhea by these androgens is discussed.

94. Effective Control of the Surgical Menopause by Estradiol Implantation at the Time of Oophorectomy and Total Hysterectomy.

Herbert S. Kupperman, Robert W. Delaplaine (by invitation) and *John R. Bottomy* (by invitation).

Departments of Therapeutics and of Obstetrics and Gynecology, New York University-Bellevue Medical Center, and Obstetrical and Gynecological Service of the Third Surgical Division (New York University) Bellevue Hospital, New York, N. Y.

Pellet implantation of steroid hormones is a widely used procedure and one employed in clinical entities requiring prolonged and continued therapy. Interest in the administration of gonadal hormones in pellet form in the female has waned, except in the sporadic treatment of the climacteric. Implantation of estrogen pellets in the surgical castrate or in a patient undergoing a spontaneous menopause has usually resulted in the initiation of irregular and at times prolonged menstrual flow. To surmount this untoward reaction, pellets of estradiol were implanted into the rectus muscle of a group of patients 45 years in age or less, at the time that the incisional wound was being closed following a total hysterectomy and bilateral oophorectomy. A series of 58 pellet-implanted patients and 58 control patients were studied. They were evaluated both by means of the menopausal index and vaginal smears. The menopausal index represented the sum of a numerical conversion of the severity of the 11 most common menopausal complaints noted in the climacteric patient. A greater than 50 per cent follow-up was obtained in the control and pellet-implanted patients. When three 25-mg. pellets of estradiol were implanted, the patient rarely experienced menopausal symptoms. The postoperative course was associated with little or no discomfort as compared to the multiple menopausal complaints observed in most control patients. Evidence from analysis of the menopausal index and vaginal smear indicated that the estrogenic activity of the pellets was maintained for at least forty-eight to fifty-four weeks. Administration of pellets at the time of surgery, before menopausal symptoms are permitted to develop after ovariectomy and total hysterectomy, is an effective and convenient method for controlling the climacteric symptoms of the surgical patient.

95. Clinical Effectiveness of a Diethylstilbestrol-Methyltestosterone Mixture in the Climacterium.

A. M. Shearman and *Thomas H. McGavack.*

New York Medical College, Metropolitan Hospital Research Unit, New York, N. Y.

A diethylstilbestrol-methyltestosterone mixture in a ratio of 1:20 has been used in a study of the climacterium in women. An evaluation of such a mixture has been based upon its effectiveness in relieving the symptom-complex of the menopausal syndrome as compared with the effectiveness of either diethylstilbestrol or methyltestosterone alone.

In all, 29 subjects have participated in this study. Every subject has received two or all three of the preparations mentioned, in relation to suitable control periods on placebo. The administration of each preparation has been continued until no further improvement was observed. The average period necessary for such a therapeutic response was about four to five weeks. The degree of improvement has been measured, whenever possible, by recording the variations in the frequency of flushes. Serial vaginal smears have also been employed.

In the present study, the estrogen-androgen combination was associated with complete remission of symptoms in 23 per cent of subjects, as compared with 26 per cent complete remission with diethylstilbestrol alone and 12 per cent with methyltestosterone alone. The degree of improvement achieved for the group on the estrogen-androgen mixture was 83 per cent as compared with a similar degree of relief obtained by 60 per cent of subjects on estrogen alone and 63 per cent of subjects on androgen alone.

96. An Intensive Three-Day Course of Stilbestrol in the Treatment of Anovulation.

Abner I. Weisman.

New York Biologic Research Foundation, Inc., New York, N. Y.

Thirty-nine anovulatory patients, complaining of infertility, were subjected to a three-month course of intensive stilbestrol therapy. Treatment consisted of 75 mg. daily of the estrogen, on days 8, 9 and 10 of the proposed new menstrual cycle. From the results obtained, the effect of such impact estrogen administration substantiated the hypothesis of Brown and Bradbury, *i.e.*, that initial large doses of stilbestrol can evoke a gonadotropic response from the pituitary gland, in certain selected cases. Of 23 anovulatory women who had been previously shown to have a proliferative or hyperplastic follicular endometrium, subsequent progestational (secretory) changes were induced in 18. Of this latter number, 12 later became pregnant. Of 16 anovulatory patients suffering from an atrophic type follicle-phase endometrium and prolonged amenorrhea, no evidence of progestational change was manifest in the endometrial biopsy specimens and no conceptions followed, even with increased amounts of stilbestrol.

97. The Effects of a Single Preovulatory Injection of Progesterone on Indices of Ovulation in Women.

Irving Rothschild and *Nan Kyung Koh* (by invitation).

Department of Obstetrics and Gynecology, Ohio State University and the Columbus State School, Columbus, Ohio.

The assumption that progesterone inhibits, and possibly induces ovulation in women is being studied. To date, 5 to 8 menstrual cycles (with data on bleeding periods, basal body temperature (BBT) curves, vaginal smears) in each of 14 adults were observed. One progesterone injection per cycle was given as follows: I—(11 subjects) 10 mg., 6 to 11 days; II—(5 subjects) 10 mg., 1 to 6 days; III—25 mg., 1 to 7 days—before the expected BBT rise. Good agreement was obtained between cycle age in days, and degree of vaginal cornification. No differences from control cycles were seen in group I. In group II, 1 delay in, and 1 absence of, a sustained BBT rise occurred, indicating delay and inhibition of ovulation, respectively. Post-progesterone bleeding appeared in 2 subjects (in 1, associated with the delay in ovulation). In group III, 2 subjects showed inhibition of, and 3, delay in ovulation, with marked lengthening of the cycle in 2 of the latter. Post-progesterone bleeding appeared in 1 girl (with no other change) and in

4 of the 5 girls showing ovulation delay of inhibition. Although these criteria are obviously inadequate for determination of ovulation induction, suggestive progesterone-induced ovulations appeared in groups I, II (1 subject), and III (4 subjects).

98. The Therapeutic Use of Pregnandiol.

George P. Heckel.

University of Rochester, School of Medicine & Dentistry, Rochester, N. Y.

To test the hypothesis that symptoms of the menopause, premenstrual distress and certain other disorders related to ovarian function are caused by endogenous allergy (altered reaction) to steroid substances, pregnandiol has been used for the treatment of these conditions. This steroid, the excretion product of progesterone and without known biologic activity, is the one which most frequently elicited reactions in women having these ovarian disorders, who were skin-tested with steroids (Heckel, G. P.: *Surg. Gynec. & Obst.* **92:** 191, 1951). Pregnandiol for hyposensitization was given in microcrystalline suspension subcutaneously in doses of 0.1 mg., or less and orally in doses of 1.0, 0.5, or 0.1 mg. Ninety-two (70 per cent), of 132 cases were improved. Symptoms were initially aggravated or induced in 43 (33 per cent). Of the 92 who were benefited, 22 were initially made worse but later improved when the dose was changed.

99. Improved Spermatogenesis after Intensive Liver Regimen With and Without Testosterone in the Therapy of Oligospermia.

S. J. Glass and *Murray Russell* (by invitation).

Cedars of Lebanon Hospital, Los Angeles, Calif.

Inasmuch as the liver participates in the metabolism of the gonadal hormones which are essential to normal gametogenesis, and because both experimental and clinical evidence have shown that fertility may be impaired in the presence of liver damage, it was deemed rational to apply a so-called "liver" regimen to the therapy of oligospermia. Previous empiric trials of such treatments had been beneficial in this clinic. Accordingly after clinical study including liver-function testing, repeated seminal analysis, testicular biopsy and determination of the urinary FSH, 15 male subjects underwent an intensive "liver" regimen for from six to eighteen months. Some of these subjects failing to show appreciable improvement or optimum levels of spermatogenesis after three to six months were given oral or parenteral testosterone on the assumption that under certain circumstances testosterone may be specifically indicated after suitable priming with a "liver" regimen. Improved spermatogenesis in terms of count, motility and morphology was attained in all except one of the subjects. Among the wives, 2 pregnancies were achieved. The fertility index of the remaining men might have been greater, were it not for the co-existence of factors impairing fertility in the female partners. Regression in spermatogenesis followed the neglect or omission of the "liver" regimen, suggesting that high concentrations of certain nutritional supplements are a prerequisite to spermatogenesis.

100. Male Pseudohermaphrodism: A Familial Occurrence.

Robert W. Schneider and *Ray Van Ommen.*

Cleveland Clinic, Cleveland, Ohio.

The occurrence of 5 cases of male pseudohermaphrodism in one family is the basis of this report. The similarity in clinical findings in these subjects, as well as the proven

cases in the literature, is striking and should permit the regular preoperative diagnosis of the condition.

Because the diagnosis was correctly anticipated prior to surgery, urinary assays for 17-ketosteroids and follicle-stimulating hormone were accomplished prior to surgery in 1 case. Hormonal assays were obtained in 2 additional cases.

The significant clinical features consist of a feminine habitus, absent axillary and pubic hair, primary amenorrhea, normal external genitalia, a blind vaginal pouch, absence of cervix and uterus, and intra-abdominal testes. The estrogen-like behavior of such testes is discussed. The cases are well illustrated.

101. Relationship of Ascorbic Acid to Adrenocortical Function in Guinea Pigs.

Albert B. Eisenstein and *Robert E. Shank* (introduced by Cyril M. MacBryde).

Nutrition Laboratories, Department of Preventive Medicine, St. Louis, Mo.

Many different observations have indicated that ascorbic acid may have an important role in the function of the adrenal cortex. This interrelationship has been the subject of a number of recent investigations. A study was undertaken in which the ability of the adrenal cortex to respond to the stimulus of injected ACTH was determined in scorbutic guinea pigs and in guinea pigs receiving 0.6 mg. and 10.0 mg. of ascorbic acid daily. The changes in body weight, eosinophil counts, adrenal weight and concentrations of ascorbic acid in the liver and adrenals were determined in control animals and in others receiving 2 mg. of ACTH daily. The period of survival of scorbutic animals was significantly prolonged when ACTH was injected. With induction of scurvy the circulating eosinophils decreased markedly. ACTH administered to scorbutic animals produced a prompt drop in eosinophil counts. Increase in adrenal weight gave evidence of adrenocortical hypertrophy in animals with scurvy. Concentrations of ascorbic acid in the adrenals and in the liver varied directly with levels of intake. These observations seem to indicate that ascorbic acid is not essential to the function of the adrenal cortex in the guinea pig and is not directly concerned in synthesis and secretion of 11-oxysteroids.

102. Increased Excretion of Formaldehydogenic ("FG") Substances in the Urine of Scorbutic Guinea Pigs.

Eli M. Nadel and *John J. Schneider* (introduced by Abraham Cantarow).

National Cancer Institute, National Institutes of Health, Bethesda, Md., and the Departments of Biochemistry and Experimental Medicine, Jefferson Medical College, Philadelphia, Pa.

The excretion of "FG" substances in the urine of normal and scorbutic guinea pigs was studied, using a modification of the method of Daughaday *et al.* (*J. Clin. Endocrinol.* **8:** 166, 1948). Animals on the vitamin C-free diet which was used, lost weight by the fifteenth day, were moribund by the thirtieth day, and showed morphologic evidence of severe scurvy on autopsy examination. Normal animals excreted an average of 100 gamma of "FG" substances daily. No significant difference was noted in the "FG" titer of guinea pigs maintained on the vitamin C-free diet for fifteen days, but by the twentieth day the titer rose to over 500 gamma. Vitamin C thus does not appear to be necessary for the elaboration and excretion of "FG" substances by the guinea pig. Rather, it appears that scurvy may function as a "stress" agent. In addition, it has been demonstrated that these "FG" substances are largely ketonic in nature and may be

almost completely extracted from benzene with water. These properties are characteristic of highly oxygenated corticosteroids.

103. The Effect of Alcohol on the Adrenal Ascorbic Acid and Cholesterol of the Rat.
James J. Smith.

Department of Medicine, New York University-Bellevue Medical Center, New York, N. Y.

Evidence of disturbances in pituitary and adrenal cortical function in alcoholics prompted investigation of the direct effect of alcohol on these glands. To this end a study was made of the adrenal ascorbic acid and cholesterol values in normal and hypophysectomized adult white rats after administration of alcohol. Sixty-three rats were given alcohol by stomach tube in doses ranging from the equivalent of 0.6 cc. to 2.1 cc. of absolute ethyl alcohol, and were sacrificed from one to seven hours later. It was found that the larger the dose of alcohol given, the greater the drop in adrenal ascorbic acid as compared with 26 control rats: the ascorbic acid drop was 29 per cent with 0.6 cc. of alcohol and 48 per cent with 2.1 cc. of alcohol. To determine the ascorbic acid drop caused by manipulation, 31 rats were given saline by stomach tube. Subtracting this factor, there was a net drop of 39 per cent in adrenal ascorbic acid in rats given 2.1 cc. of alcohol. Adrenal cholesterol on this maximal dose fell 45 per cent, with a net drop of 40 per cent. Similar studies on hypophysectomized rats showed average adrenal ascorbic acid values of 14 rats given saline by stomach tube to be 265 mg. per cent, and of 12 rats given the equivalent of 1.1 cc. of absolute alcohol by stomach tube, 263 mg. per cent. This represents an insignificant net drop of 0.8 per cent. Similarly, the cholesterol values did not change. Thus, it appears that in the adult white rat alcohol stimulates the adrenal cortex, as indicated by a significant drop in adrenal ascorbic acid and cholesterol. This does not occur in the hypophysectomized rat.

104. Maternal and Fetal Adrenal Cortical Interrelationships.
J. M. Jones (by invitation), *C. W. Lloyd*, and *T. C. Wyatt* (by invitation).

Departments of Pediatrics and Obstetrics, State University of New York Medical Center at Syracuse, N. Y.

The effect of stimulation of the maternal adrenal cortex on the fetal adrenal has been studied. In all cases, maternal and fetal cholesterol were determined. In some cases ascorbic acid was also determined. Cholesterol concentrations of the adrenals of unstimulated pregnant animals averaged about 45 per cent lower than in the nonpregnant animals. Fetal adrenal cholesterol was low (0.8 mg./100 mg.). Exposure to cold for a period sufficient to cause a decrease of 63 per cent in the adrenal cholesterol content of nonpregnant animals caused a decrease of 46 per cent in the adrenal cholesterol of pregnant animals. The decrease in maternal adrenal cholesterol was accompanied by an increase in fetal cholesterol averaging 55 per cent, which was proportionate with the maternal response. A similar rise in fetal adrenal cholesterol occurred when the maternal adrenal was stimulated by ACTH.

105. Some Factors Involved in the Assay of Adrenal Cortical Hormones by the Mouse Eosinopenic Procedure.
Robert S. Speirs (introduced by Dr. R. K. Meyer).

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

A method of assaying adrenal cortical hormones based upon a decrease in the number

of circulating eosinophils of epinephrine-pretreated adrenalectomized mice has been developed. The eosinopenic response during a three-hour period was found to be specific to the hormones of the adrenal cortex (Speirs and Meyer, 1949 and 1951). The entire assay can be performed within twelve hours and is easily adaptable for screening large numbers of organic compounds for cortisone-like activity, or for assaying blood, urine and tissue extracts for adrenal cortical hormones.

Two strains of mice have been found to be extremely sensitive to the 11-oxycorticosteroid hormones: the Jax C_{57} Brown and the Jax C_{57} Black mice. Hybrid mice, produced by a cross between the two strains have the same sensitivity but are more resistant to stress and toxic materials. In male mice, weighing between 20 and 25 grams, a straight-line log-dose-response was obtained following injections of between 0.5 and 6 micrograms of cortisone acetate, cortisone (free alcohol), compound F, and compound F acetate. Pooled pregnancy urine, and urine from patients receiving ACTH therapy were found to be high in eosinopenic activity. This material was extractable in chloroform and ethylene chloride and could be destroyed by boiling in an acid medium (pH2).

106. Adrenal Cortical Function at Death as Measured by the Level of Circulating Eosinophils.

Paul B. Jennings. (introduced by Matthew Molitch).

Atlantic City Hospital, Atlantic City, N. J.

Since the adrenal cortex plays such a vital role in the maintenance of life, especially during stress, it was felt that it would be of interest to evaluate adrenal cortical function in patients who are moribund as a result of various types of stress, in order to see if adrenal failure may play a role in the process of death. Selye noted pathologic changes in the adrenals during the exhaustion stage of the alarm reaction but reached no conclusions as to the eosinophil trend.

A controlled series of about 50 patients with a variety of diseases was followed until their death, using serial eosinophil counts as an index of cortical function. In the vast majority of cases, the count fell to zero or close to zero and remained so until death. Suddenness of death seemed to be a factor in those few in whom a marked eosinopenia did not develop. The eosinophils rose to normal in those patients who did not succumb.

This study would indicate that 1) the adrenals do not fail under fatal stress but continue to function up to the very moment of death, and 2) the eosinophil level may be of prognostic significance.

107. Inability of ACTH and Cortisone to Alter a Physiologic Human Hypersensitivity Regularly Abolished by Pregnancy.

William Q. Wolfson, William D. Robinson (by invitation) and *Ivan F. Duff* (by invitation).

From the Rackham Arthritis Research Unit, University of Michigan, Ann Arbor, Mich.

ACTH and cortisone are beneficial in many of the allergic and rheumatic diseases which may remit spontaneously during pregnancy or hepatitis. Many have wondered whether increased glycocorticoid secretion underlies these spontaneous remissions but no real evidence yet supports this hypothesis. The normal nonpregnant human adult shows a physiologic hypersensitivity to the intradermal injection of human colostrum marked by a rapidly appearing wheal and flare. This intolerance is

regularly lost early in pregnancy and its disappearance forms the basis of the Falls-Freda pregnancy test. Possible glycocorticoid effects were studied in 12 nonpregnant subjects receiving prolonged treatment with ACTH (av. 102 mg./day) or cortisone (av. 167 mg./day). The normal nonpregnant hypersensitive reaction to colostrum was not diminished or absent in any subject. Colostrum sensitivity represents a hypersensitive reaction abolished by pregnancy but not by therapeutically adequate dosage of ACTH or cortisone. This observation suggests that anti-allergic factors other than increased glycocorticoid secretion may be partly responsible for remissions in hypersensitivity disorders during pregnancy.

108. Metabolic and Cardiovascular Effects of Various Steroids Alone and in Combination.

G. M. C. Masson (by invitation), *A. C. Corcoran* and *Irvine H. Page.*

Research Division, Cleveland Clinic Foundation, Cleveland, Ohio.

Sixty-six uninephrectomized female rats, receiving 1 per cent saline were divided into groups as follows: 1) control, 2) desoxycorticosterone acetate (DCA), two 35-mg. pellets subcutaneously, 3) DCA and cortisone acetate, 4) Lipo-adrenal Extract (Upjohn), 18 rat units daily, 5) Lipo-adrenal Extract and DCA, 6) 17-hydroxy-11-desoxycorticosterone acetate (compound S), 7) pregnenetriolone diacetate, 8) pregnenetriolone diacetate and DCA, 9) cortisone acetate, 10) 17-hydroxyprogesterone, 11) 17-hydroxyprogesterone and DCA. Steroids other than DCA were given subcutaneously in doses of 2.5 to 5 mg. daily, as water suspensions. The experiments were continued for thirty days.

Diuresis was greatest in group 3, less in the DCA-treated groups and slight in group 6. Arterial pressure increased in the DCA groups, without inhibition or potentiation by steroids other than cortisone. Group 3 manifested early onset of hypertension, hematuria and proteinuria, weight loss, edema and a high mortality during the experimental period. Cortisone alone caused weight loss only. Among results of concurrent metabolic and chemical studies, the most novel was an increase in the thiobarbituric acid index of cerebral tissue in the cortisone and Lipo-adrenal groups.

109. The Effects of Adrenocortical Hormones on the Distribution and Kinetics of Body Water.

I. S. Edelman (by invitation), and *D. L. Wilson* (by invitation) (introduced by G. W. Thorn).

Harvard Medical School and Peter Bent Brigham Hospital, Boston, Mass.

Following the rapid intravenous injection of D20, a characteristic arterial time-concentration curve is obtained. When analyzed on a semi-log plot, the pre-equilibrium portion of this curve resolves into two straight-line components, whose slopes are a function of cell permeability. In 4 normal male subjects, total body water (61 per cent body weight) was calculated from the equilibrium concentrations, and effective extracellular compartments (21 per cent of extracellular water exchanged per minute with intracellular water), from the slopes of the pre-equilibrium straight-line components. Data were obtained before and after treatment of 1 normal subject with 650 mg. of ACTH for five days, of 1 male Addisonian patient with 1.2 Gm. of cortisone over twelve days, and 1 male rheumatoid arthritic patient with 6.0 Gm. of cortisone over

fifteen days. In this group, there was a mean weight gain of 2.3 Kg. and a mean gain in total body water of 2.7 liters, of which 35 per cent (1.0 liter) was extracellular. Data on internal rates of water exchange are presented.

110. Further Studies on Hormonal Control of Serum Lipid Partition.

David Adlersberg, Stanley R. Drachman (by invitation) and *Louis E. Schaefer* (by invitation).

Mount Sinai Jospital, New York, N. Y.

Evidence is increasing that in addition to the thyroid and anterior pituitary, the adrenal cortex may have an important function in the hormonal regulation of lipid metabolism. Previous studies of total and esterified serum cholesterol, phospholipid and neutral fat revealed well defined changes in patients treated with cortisone or ACTH for a variety of pathologic conditions. These observations were continued over prolonged periods of time and a study of the interrelations of the serum lipids was undertaken.

The changes in the serum lipids may be summarized, generally, as a rise in cholesterol (total and esterified), often resulting in sustained hypercholesteremia with protracted therapy; an approximately parallel elevation of phospholipid; a diphasic alteration of neutral fat: a precipitous but transient fall in the first two to three weeks of therapy and later a gradual elevation toward or above pretreatment levels; turbidity of "fasting" sera was observed, even at normal levels of neutral fat, suggesting changes in the relative concentration of the lipid fractions or an alteration in the ratio of free and conjugated lipids (lipoproteins). The interrelations of the serum lipid components before, during, and after hormonal therapy remained within the normal range; there was nevertheless a consistent alteration noted. A lowering of the free/total cholesterol ratio was seen in almost every instance. A less marked rise in the total cholesterol/phospholipid ratio was due to a disproportionate elevation of total cholesterol. The ratio of free/total cholesterol:phospholipid showed a consistent reduction. A change in the rate of esterification of cholesterol and synthesis of phospholipid under the influence of cortisone and ACTH is suggested. Animal experiments with larger doses of these hormones are in progress.

111. Clinical Observations on the Effects of Cortisone and ACTH on the Peripheral Vascular Bed.

M. James Whitelaw.

Humanity Memorial Hospital, Phoenix, Ariz.

The following observations made by the author tend to indicate that both ACTH and cortisone cause a definite increase in the peripheral vascular bed:

1. The reduction of hyperpyrexia following diaphoresis, the latter presumably being brought about by peripheral vasodilatation.
2. The marked plethora in Cushing's syndrome as well as the changes in the color of the nail beds in aplastic and target cell anemia without any demonstrable change in the blood picture.
3. The disappearance of cyanosis and anesthesia, and return of warmth to the injured extremity in cold injury.
4. The rapid epithelialization and repair of the dermis when involved in thermal or cold injuries.

It is postulated that some of the beneficial effects of ACTH and cortisone may be based on this phenomenon. Therefore, it is suggested that it may be of benefit in those specific diseases where there is circulatory impairment of the peripheral vascular bed.

112. Observations on the Use of Oral Cortisone and ACTH in the Open Treatment of Burns.

M. James Whitelaw.

Humanity Memorial Hospital, Phoenix, Ariz.

This study includes 3 patients with severe second-degree burns of the face, neck and arms.

No therapy except the hormones was given. Two patients received 100 mg. of ACTH daily for approximately one week, after which one of these was given 80 mg. in divided doses daily. The third case received 300 mg. of oral cortisone the first day and 200 mg. in divided doses daily thereafter.

In all 3 cases, within the first twenty-four hours after institution of therapy, it was noted that the blebs and edema had undergone marked regression. There was no pain, toxicity or suppuration. The patients were all ambulatory and able to feed themselves after the second day. Desquamation commenced on the third day. In 2 patients (1 of whom received cortisone and 1, ACTH) re-epithelialization was complete within seven days after the initial injury. The third case, treated with ACTH, was discharged in three weeks.

Oral cortisone appeared to be just as effective as ACTH in this type of burn.

113. The Effects of ACTH on Scorpion Neurotoxin.

M. James Whitelaw and *Herbert Stahnke* (by invitation).

The Poisonous Animal Research Laboratories of the Arizona State College, Tempe, Ariz.

Pooled venom of the scorpion (*Centruroides sculpturatus*) which had been collected by electrical stimuli was standardized on rats by the method of Stahnke.

Three rats were given intramuscular ACTH in varying dosages of 2.0, 4.0 and 8.0 mg., and simultaneously 1 MLD of scorpion venom. Three other groups of rats were given the same dosages of ACTH, but the venom was given half an hour, one hour and four hours, respectively, after the ACTH.

The time of the original convulsions and the subsequent clinical phenomena were compared with those of the controls. In every case convulsions and death took place earlier in the animals treated with ACTH. In no case was the clinical course altered by the use of the hormone. Autopsies on the rats who received the drug revealed no essential differences in the gross pathology as compared with those who received none.

It is concluded that ACTH imparts no protection to rats from the neurotoxin of *Centruroides sculpturatus* and may, if anything, hasten death. These results suggest that it should not be used in the treatment of scorpion stings.

114. The Relation Between Basal Metabolic Rate and 17-Ketosteroid Output in Rheumatic Patients Under the Influence of Cortisone.

Manuel M. Villaverde and *Luis Pérez Montes* (by invitation).

Habana, Cuba.

The B.M.R. was found to be either normal (10 cases) or low (8 cases) among 20

rheumatic patients, according to the normal rates found in tropical countries (normal range: minus 15 per cent to plus 5 per cent).

When the B.M.R. was low (9 cases) the urinary excretion of 17-ketosteroid was normal in 5, low in 3, and high in 1. When the 17-ketosteroid output was low (10 cases) the B.M.R. was normal in 5, low in 3, and high in 2. Thus, they vary inversely.

Sixteen cases were studied during cortisone therapy, and 8 showed increased B.M.R. Five of them previously had a low B.M.R., and 3, normal. In 4 cases the B.M.R. did not vary and in the other 4, it decreased. Thus, cortisone therapy increased the B.M.R. particularly when it was previously low.

When both B.M.R. and 17-ketosteroid output are low, it probably is due to the influence of hypothyroidism. But most of hypometabolic rheumatic patients have a low B.M.R. because of a decreased adrenal function.

115. The Inhibition of Adrenal Function by Cortisone, in Rheumatic Patients.

Manuel M. Villaverde (with the technical assistance of J. Lopez Rendules and his associates, J. Angora, M. Gutiérrez, J. Melman, and Eulalio Martin).

Habana, Cuba.

Cortisone produces a decrease of 17-ketosteroid output, except when there is a low initial excretion, according to most authors. We believe that even in these cases cortisone inhibits the adrenal function.

Most of our rheumatic patients have weak adrenal bodies, because only 1, among 17 patients, produced large amounts of urinary 17-ketosteroid. The rest produced either normal (9 cases) or low (6 cases) amounts.

Cortisone produced increased 17-ketosteroid output in 8 patients (6 had initial low excretion; 2, normal). No variation occurred in 5 cases (4 with previous normal excretion). According to the constitutional type, the increase occurred in those patients corresponding to types with more strong adrenals.

The actual excretion of 17-ketosteroid in patients injected with cortisone must be the sum of the steroids produced within the body plus the metabolic residues of the injected substance. If we subtract the amount that may represent the excretion of the injected steroid, 58.8 per cent of the patients eliminated less than the corresponding average during cortisone therapy.

Thus, we must agree with the hypothesis of the inhibition of the adrenal function by cortisone, in spite of some contradictory data.

116. A Patient With Cushing's Syndrome: Metabolic Effects of Potassium Acetate, Adrenocorticotropin, Cortisone, Methyltestosterone and Desoxycorticosterone.

Grant W. Liddle (by invitation), *Leslie L. Bennett* and *Joseph E. Giansiracusa* (by invitation) (with the technical assistance of Donald Island and John Whitney).

Divisions of Medicine and Physiology, University of California School of Medicine, San Francisco and Berkeley, Calif.

Observations were made in a 35-year-old woman with Cushing's syndrome, presenting amenorrhea, "buffalo" obesity, hypertension, acne, hirsutism, muscular weakness, diabetes, erythrocytosis and elevated urinary 17-ketosteroids. X-ray examination revealed spherical enlargement of the sella but failed to demonstrate adrenal enlargement.

Balance studies were done over a three-month period. Ingestion of 36 Gm. of potassium acetate daily was associated with an increase of sodium and a decrease of phosphate in the urine. Administration of 100 mg. of ACTH per day was associated with a fall in circulating eosinophils, a rise in fasting blood sugar and an increase of urinary sugar, nitrogen, sodium, uric acid and 17-ketosteroids. Administration of 100 mg. of cortisone daily was associated with a fall in circulating eosinophils, a slight diuresis of sodium and, following cortisone withdrawal, a slight (? significant) reduction of urinary 17-ketosteroids. Administration of 300 mg. daily of Δ-5-pregnenolone was associated with diminution of urinary 17-ketosteroids. Administration of 100 mg. of DCA daily was associated with sodium retention, a rise in circulating eosinophils and a fall in urinary uric acid. Methyltestosterone (100 mg. daily) produced a markedly positive nitrogen balance and a delayed reduction of urinary 17-ketosteroids. It is postulated that suppression of endogenous ACTH production accounts for many metabolic changes following administration of the aforementioned steroids.

117. Evidence for a "Salt-Losing" Adrenal Hormone in Congenital Adrenal Virilism Associated With Addisonian-Like Symptoms.

Joseph W. Jailer.

College of Physicians and Surgeons, Columbia University, New York, N. Y.

Three patients exhibiting the virilizing syndrome with associated Addisonian-like symptoms are described. Two (a female pseudohermaphrodite and her brother) occurred in one family. The syndrome is characterized physiologically by excessive production of androgens, normal corticoids and what appears to be a deficiency in DCA-like hormone(s) To explain this syndrome, Wilkins has postulated the production of an abnormal steroid with an action antagonistic to DCA.

The administration of ACTH to a 2½-year-old boy with this syndrome, who was being maintained on 5 mg. of DCA throughout the study, increased the urinary sodium excretion from 68 to over 130 mEq./day. With this, there was a decline in serum sodium from 134 to 124 mEq. This was associated with a rise in 17-ketosteroids from 12 to over 20 mg./day and a fivefold increase in formaldehydogenic steroids. Thus, in this condition, adrenal stimulation results in a marked diuresis of sodium. The administration of cortisone, on the other hand, produced an equivocal decrease in urinary sodium and a rise in serum sodium and was accompanied by a decline in the urinary 17-ketosteroid value to approximately normal values. This would indicate a decrease in adrenal secretion, presumably by endogenous ACTH inhibition. On maintenance oral doses of cortisone, it was possible to withdraw DCA and maintain the patient's serum sodium at normal values. Previously, without cortisone, cessation of DCA therapy caused a marked fall in the serum sodium.

118. Estrogen-Producing Tumors of the Adrenal: Report of Four Cases.

Edward Rose, F. C. Dohan, Harold F. Zintel (by invitation) and *John W. Eiman* (by invitation).

Hospital of the University of Pennsylvania, Philadelphia, Pa.

The incidence of estrogen-producing tumors of the adrenal is reviewed and 4 additional cases in adults are presented. Three of the patients were males, aged 20, 30 and 56 years. One patient's history suggested a previous transitory forme fruste of Cushing's syndrome with precocious puberty, but later he presented only obesity, short stature,

abnormal hair distribution, an adrenal mass, and increased urinary estrogens and 17-ketosteroids. The other male patients presented gynecomastia, hypertension and testicular atrophy with a marked increase in urinary estrogens, but normal or low 17-ketosteroids. Estrogen excretion decreased to normal levels after removal of the tumors. The fourth patient, a 49-year-old woman with a large adrenal cortical carcinoma succumbed to extensive metastases nine months after operation. She presented masculinizing features with a marked increase in urinary estrogens and 17-ketosteroids, and bizarre variations in blood sugar levels. Difficulties in the identification of cell types in adrenal neoplasms are discussed.

119. The Use of ACTH and Cortisone in the Management of Acute Abdominal Emergencies.

Lenore Boling (by invitation), *John Newkirk* (by invitation), *Phillip Baxter* (by invitation), *Sheldon Margen* and *Laurance W. Kinsell.*

Institute for Metabolic Research, and the Surgical Service, Highland Alameda County Hospital, Oakland, Calif.

The availability of potent chemotherapeutic agents has greatly simplified the management of acute infectious processes in general. Nonetheless, a generalized peritonitis still represents a serious problem.

For a period of some months, all patients admitted to Highland Hospital with known or suspected generalized peritonitis have been considered from the standpoint of advisability of ACTH and/or cortisone therapy in addition to adequate chemotherapy. Initially, only those patients who were extremely toxic and not responding well to chemotherapy were so treated. More recently, patients in a less severe category have been treated in an attempt to determine whether the period of convalescence could be shortened. The patients so treated included a number of children who had ruptured appendices, adults with ruptured appendices, and individuals with peritonitis from a variety of causes including bile peritonitis resulting from rupture of the gallbladder.

There appears to be no question that the administration of ACTH and cortisone in addition to chemotherapy to patients who are extremely toxic results in rapid disappearance of toxicity and rapid return of normal gastro-intestinal function. Patients with a generalized peritonitis but with relatively moderate systemic toxicity, usually respond well to chemotherapy alone, but may respond more rapidly to a combination of ACTH, cortisone, and chemotherapy.

From these observations and from other reported observations regarding the use of ACTH and cortisone in other severe infectious conditions, it appears that adrenal steroids interpose a blockade between the toxins resulting from infectious agents and the peripheral tissue cells, without in any way modifying the growth of the infectious agent. It, therefore, appears probable that the use of these hormones in conjunction with chemotherapeutic agents represents more optimal therapy than chemotherapy alone in many severe infectious processes.

120. Effect of Oral and Intrasplenic Administration of Cortisone.

A. M. de Andino, Jr. and *J. L. Rivero-Fontan* (introduced by Karl E. Paschkis).

Jefferson Medical College, Philadelphia, Pa.

In vitro studies in our laboratories have shown that liver and other tissues partly destroy the activity of cortisone upon incubation (*Fed. Proc.* 1951); a small amount of

biliary excretion of administered glucocorticoids has been previously observed in bile-fistula dogs (*Fed. Proc.* 1948).

In experiments reported in this paper the effect of cortisone upon thymic weight of mice was studied, comparing (a) intrasplenic and subcutaneous administration, and (b) oral (stomach tube) and subcutaneous administration. The latter route duplicates clinical therapeutic routes of administration, the former eliminates the variable of intestinal absorption. In either experiment the weight loss of the thymus offers a quantitative measurable endpoint. Control animals received 0.85 per cent NaCl by stomach tube or intrasplenically respectively, in order to eliminate effects upon the thymus of an alarm reaction, which might be induced by the procedure itself. A single intrasplenic injection of cortisone acetate or cortisone hemisuccinate was almost as effective as subcutaneous injection; a single large dose given by stomach tube was less effective than when administered subcutaneously.

Liver changes were observed following oral administration of a single large dose of cortisone.

121. An Excellently Tolerated, Heat-Stable, Long-Acting ACTH Preparation Suitable for Prolonged and Ambulatory Treatment.

William Q. Wolfson, Robert E. Thompson (by invitation), *William D. Robinson* (by invitation), *Leon Lewis* (by invitation) and *Ivan F. Duff* (by invitation).

Rackham Arthritis Research Unit, University of Michigan Medical School, Ann Arbor, Mich.; Department of Pharmacology, The Armour Laboratories, Chicago, Ill.; and Department of Medicine, Highland-Alameda County Hospital, Oakland, Calif.

In 1,177 patient-days, 29 patients received 799 doses (61 grams) of Acthar-in-oil (pork ACTH, Armour, in aluminum monostearate and sesame oil, 40 mg./ml., formerly designated Adactar-O-40.). Immediate discomfort was less than after aqueous ACTH. There were no systemic reactions and only occasional serious late local irritation. Abscesses occurred in 3 patients who together received a total of 200 injections. No abscess was sterile and all healed promptly after drainage, despite continued ACTH. Difficult therapeutic problems were deliberately selected for study. Including periods of initial and maintenance treatment, the median daily requirement was 47 mg./day (av. dev. ±25 mg./day). The median interval between doses was thirty-six hours (av. dev. ±fourteen hours). Transfer from four daily doses of aqueous ACTH to one daily dose of Acthar-in-oil somewhat increased daily requirement, but transfer to two daily doses did not. Requirements below 80 mg./day are conveniently met by single daily doses, and below 40 mg./day by less frequent administration. Acthar-in oil is easily given, stable in liquid form at room temperature, and better tolerated than other available ACTH preparations. Acthar-in-oil appears entirely suitable for prolonged ambulatory ACTH treatment and for supervised self-administration by patients.

122. Effect of Alanine, Pyruvate and Glutamate on Insulin Reaction in Mice.

George R. Fisher (introduced by Karl E. Paschkis).

Jefferson Medical College, Philadelphia, Pa.

A large body of evidence is available indicating that alanine is a "glucogenic" amino-acid. It was believed that alanine entered the carbohydrate cycle by forming pyruvic acid in the process of transamination. Recent observations cast some doubt on such a relatively simple explanation. Gurin, for example, showed that the extra glucose excreted

by phlorhizinized rats after administration of labeled alanine did not contain the labeled carbon of the amino acid.

In experiments reported in this paper, male mice were injected subcutaneously with insulin (control group I) and with insulin subcutaneously, followed by glucose intravenously (control group II).

Experimental groups received intravenous injections of *d-l* alanine, sodium pyruvate and monosodium glutamate respectively, after the subcutaneous administration of insulin. Alanine afforded protection against the insulin reaction, as did glucose, as judged by incidence and time of onset of convulsions, and by mortality. Neither sodium pyruvate nor monosodium glutamate was effective in this respect. This would suggest, that under the conditions of the experiment, alanine exerts its protective action by some mechanism other than by entering the carbohydrate cycle by way of pyruvate.

Alanine was without effect in reversing the insulin reaction in adrenalectomized mice but the effect was largely restored by the prior administration of cortisone.

123. Diabetic Effect of Insulin on Glucose Tolerance Tests in Normal Individuals. A Preliminary Report.

B. A. Watson and *D. W. Jones* (by invitation).

Metabolic Service of the Clifton Springs Sanitarium and Clinic, Clifton Springs, N. Y.

In this study to date 6 underweight but otherwise healthy individuals (5 females and 1 male) were given increasing doses of regular insulin twenty minutes before each meal. The initial dose was 5 units three times daily and was increased *each day* by 5 units over a period of fourteen to nineteen days, resulting in a total daily dosage of from 45 to 75 units of insulin at the end of the treatment period. Oral glucose tolerance tests were performed before and at the cessation of insulin therapy:

	Fasting	*½ hr.*	*1 hr.*	*2 hrs.*	*2½ hrs.*
Pre-treatment mean blood sugar values (mg. %)	92	155	139	99	82
Post-treatment mean Blood sugar values (mg. %)	106	180	226	194	169

Results revealed that a diabetic type of blood sugar curve resulted in all cases. Individual cases are shown and attention is drawn to the possibility of making diabetes more severe by the injudicious use of insulin.

124. Preadolescent Hyperantuitarism in Diabetes Mellitus.

Carlos P. Lamar.

Department of Endocrinology and Diabetes Clinics, Jackson Memorial Hospital; Chief of Staff, Mercy Hospital, Miami, Florida.

The insulin-antagonistic action of the anterior pituitary has been demonstrated by Houssay and others.

Diabetes mellitus frequently complicates states of hyperantuitarism such as acromegaly, giantism, pituitary basophilism, exophthalmic goiter (hyperthyrotropinism), and the climacteric syndromes, in which urinary levels of gonadotropins are elevated.

The well known instability of childhood diabetes may be associated with variations in the activity of the anterior pituitary, in effect, with juvenile hyperantuitarism.

This has been suggested by the following clinical observations:

1) Frequently the severe or "brittle" diabetes of childhood improves and becomes more easily manageable after puberty and growth are completed (illustrated with case histories).
2) Diabetic coma was observed in several preadolescent girls with markedly eunuchoidal appearance, relatively tall stature, and with a history of recent intense "spurts of growth." There was delay in sexual maturation with little or no sexual hair or breast growth, and infantile genitalia. Three of these patients are presented as an illustration. One of the girls required over 5,000 units of insulin for control of severe acidosis and did not show clinical response until after estrogen had been administered intensively. Two others who received estrogen at the onset of treatment for diabetic coma required relatively small amounts of insulin and recovered rapidly.

It is suggested that in brittle childhood diabetes with delayed puberty, it may be advantageous to attempt induction of sexual maturation through the careful administration of estrogens or androgens with the possibility of improving the diabetic picture.

ENDOCRINOLOGY INDEX

The Association for the Study of Internal Secretions has undertaken to prepare a comprehensive Index of the articles published in *Endocrinology*. This Index will appear in three volumes. Volume III, covering papers published in 1940 through June, 1947, in volumes 26–40 of the journal, has been completed and is now available for distribution. Volumes I and II of the Index have not yet been completed.

Volume III of the Index is available gratis to members of the Association for the Study of Internal Secretions. The price to all non-members is $2.50. Inquiries and orders should be addressed to:

Dr. H. H. Turner, Secretary
1200 North Walker Street
Oklahoma City 3, Oklahoma.

Post-Graduate Course in Pediatric Endocrinology

HARVARD MEDICAL SCHOOL

A Pediatric Post-Graduate Endocrinology Course will be held at the Burnham Memorial Hospital for Children, Massachusetts General Hospital, Boston, under the direction of Dr. Nathan B. Talbot and associates, October 1 through October 6, 1951, daily from 9:00 A.M. to 4:30 P.M. For further details, write Courses for Graduates, Harvard Medical School, 25 Shattuck Street, Boston, Mass.

Biological Photographic Association, Inc.

The Biological Photographic Association will hold its Twenty-First Annual Meeting in Boston, Mass., September 12 through 14, 1951. The Chairman of the Boston Chapter is Ferdinand R. Harding, Children's Medical Center, Boston; and the Secretary is Laurence B. Brown, Harvard School of Dental Medicine, 188 Longwood Avenue, Boston, Mass.